# 0026-895X/91/061006-8\$03.00/0 Copyright © by The American Society for Pharmacology and Experimental Therapeutics All rights of reproduction in any form reserved. **MOLECULAR PHARMAcoLoGY, 40:1006-1013 Halothane Inhibits Agonist-induced Inositol Phosphate and Ca2 SORE-BOEX/D1/081006-8403.00/0**<br>Computation in any form reserved<br>All rights of reproduction in any form reserved<br>Discussion Phanometers, 40:1005-1013<br>Signaling in A7r5 Cultured Vascular Smooth Muscle Cells<br>Signaling in A7r **Signaling in A7r5 Cultured Vascular Smooth Muscle Cells**<br>J. CHRISTOPHER SILL, CINDY UHL, SCOTT ESKURI, RUSSELL VAN DYKE, and JAMES TARARA

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# **SUMMARY**

**EUMMARY**<br>Halothane, an anesthetic with marked depressant effects on the only<br>circulation, was studied for its ability to inhibit inositol phosphate is m **SUMMARY**<br>Circulation, an anesthetic with marked depressant effects on the<br>circulation, was studied for its ability to inhibit inositol phosphate is<br>and Ca<sup>2+</sup> signaling evoked by the vasoactive hormone arginine ha **SUMMARY**<br>Halothane, an anesthetic with marked depressant effects on the<br>circulation, was studied for its ability to inhibit inositol phosphate<br>and Ca<sup>2+</sup> signaling evoked by the vasoactive hormone arginine<br>vasopressin (AV Halothane, an anesthetic with marked depressant effects on the circulation, was studied for its ability to inhibit inositol phosphate and  $Ca^{2+}$  signaling evoked by the vasoactive hormone arginine vasopressin (AVP) and rial<br>outline, an arestretic with marked depressant effects on the circulation, was studied for its ability to inhibit inositol phosphat<br>and Ca<sup>2+</sup> signaling evoked by the vasoactive hormone arginin<br>vasopressin (AVP) and C circulation, was studied for its ability to immote inostic prospirate is<br>and  $Ca^{2+}$  signaling evoked by the vasoactive hormone arginine has<br>vasopressin (AVP) and  $Ca^{2+}$  responses elicited by platelet-de-<br>arived growth f and Ca<sup>21</sup> signaling evoked by the vasoactive hormone argininy<br>asopressin (AVP) and Ca<sup>2+</sup> responses elicited by platelet-derived growth factor and by thapsigargin in cultured A7r5 vascula<br>smooth muscle cells. Changes in vasopressin (AVP) and Ca<sup>-1</sup> responses elicited by platelet-de-<br>rived growth factor and by thapsigargin in cultured A7r5 vascular<br>smooth muscle cells. Changes in apparent  $[Ca^{2+}]$ , were meas-<br>ured using the indicator indorived growth ractor and by thapsigargin in cultured A715 vasculations and column scheens. Changes in apparent  $[Ca^{2+}]$ , were measured using the indicator indo-1 and flow cytometry, whereas inositol phosphate levels were de since of this constrained in applement [Ca<sup>2</sup>], were measured using the indicator indo-1 and flow cytometry, whereas<br>inositol phosphate levels were determined using myo-[<sup>3</sup>H]inositol<br>and column chromatography. Preincubat inositol phosphate levels were determined using *myo*-[<sup>3</sup>H]inositol by<br>and column chromatography. Preincubation with clinically rele-<br>want concentrations of halothane resulted in dose-dependent als<br>depression of [Ca<sup>2+</sup>], and column chromatography. Preniculoation with clinically relevant concentrations of halothane resulted in dose-dependent depression of  $[Ca^{2+}]$ , responses evoked on stimulation with AVP. Halothane (2.0%) inhibited the in vant concentrations of halothane resulted in loose-dependent also<br>depression of  $[Ca^{2+}]$ , responses evoked on stimulation with AVP. to<br>Halothane (2.0%) inhibited the increases in  $[Ca^{2+}]$ , by 34-45%. tidd<br>in cells incubat depression of [Ca<sup>---</sup> j<sub>i</sub> responses evoked on sumulation with AVP.<br>Halothane (2.0%) inhibited the increases in  $[Ca^{2+}$ <sub>1</sub>, by 34-45%.<br>In cells incubated in Ca<sup>2+</sup>-free medium plus 0.5 mm ethylene<br>glycol bis(*ß*-aminoet malouriane (2.0%) inhibited the increases in  $[Ca^{2+}]$ , by 34–43%. The cells incubated in Ca<sup>2+</sup>-free medium plus 0.5 mm ethylene splycol bis( $\beta$ -aminoethyl ether)- $N, N, N', N'$ -tetraacetic acid, the hather bothane effect w In cells includated in Ca<sup>21</sup>-iree medium plus 0.5 mm emyene spot glycol bis( $\beta$ -aminoethyl ether)-N,N,N',N'-tetraacetic acid, the hatiothane effect was more marked, with 1.5% halothane inhibiting The the responses by ap

only 15%, suggesting that release of Ca<sup>2+</sup> rather than Ca<sup>2+</sup> influx is more sensitive to inhibition by the anesthetic. The effects of only 15%, suggesting that release of Ca<sup>2+</sup> rather than Ca<sup>2+</sup> influx<br>is more sensitive to inhibition by the anesthetic. The effects of<br>halothane on Ca<sup>2+</sup> homeostasis are not explained solely by only 15%, suggesting that release of Ca<sup>2+</sup> rather than Ca<sup>2+</sup> influx<br>is more sensitive to inhibition by the anesthetic. The effects of<br>halothane on Ca<sup>2+</sup> homeostasis are not explained solely by<br>anesthetic-induced deplet only 15%, suggesting that release of Ca<sup>2+</sup> rather than Ca<sup>2+</sup> influx<br>is more sensitive to inhibition by the anesthetic. The effects of<br>halothane on Ca<sup>2+</sup> homeostasis are not explained solely by<br>anesthetic-induced deplet only 15%, suggesting that release of Ca<sup>2+</sup> rather than Ca<sup>2+</sup> influx<br>is more sensitive to inhibition by the anesthetic. The effects of<br>halothane on Ca<sup>2+</sup> homeostasis are not explained solely by<br>anesthetic-induced deplet only 15%, suggesting that release of Ca<sup>-1</sup> rather than Ca<sup>-1</sup> limitx<br>is more sensitive to inhibition by the anesthetic. The effects of<br>halothane on  $Ca^{2+}$  homeostasis are not explained solely by<br>anesthetic-induced deple Is more sensitive to inhibition by the anesthetic. The emects of<br>halothane on Ca<sup>2+</sup> homeostasis are not explained solely by<br>anesthetic-induced depletion of Ca<sup>2+</sup> from intracellular stores,<br>because the anesthetic inhibite malouriane on Ca<sup>21</sup> homeostasis are not explained solety by<br>anesthetic-induced depletion of Ca<sup>2+</sup> from intracellular stores,<br>because the anesthetic inhibited increases in  $[Ca^{2+}]$ , elicited by<br>thapsigargin in cells susp anesmetic-induced depietion or Ca<sup>--</sup> from intracellular stores,<br>because the anesthetic inhibited increases in  $[Ca^{2+}]$ , elicited by<br>thapsigargin in cells suspended in  $Ca^{2+}$ -free medium by only<br>31%. Halothane inhibited because the anesthetic inhibited increases in [Ca<sup>-1</sup>], encited by thapsigargin in cells suspended in Ca<sup>2+</sup>-free medium by only 31%. Halothane inhibited inositol phosphate formation elicited by AVP, suggesting an additio thapsigargin in cells suspended in Ca<sup>---</sup>-free medium by or<br>31%. Halothane inhibited inositol phosphate formation elicite<br>by AVP, suggesting an additional means by which the anesther<br>may alter agonist-induced Ca<sup>2+</sup> respo  $51\%$ . Halouraine inhibited inostic prosphate formation elictied<br>by AVP, suggesting an additional means by which the anesthetic<br>may alter agonist-induced Ca<sup>2+</sup> responses. The current results<br>also demonstrate that haloth by AVP, suggesting an additional means by which the anesthetic<br>may after agonist-induced Ca<sup>2+</sup> responses. The current results<br>also demonstrate that halothane actions are not specific solely<br>to responses evoked by AVP, wh also demonstrate that halothane actions are not specific solely<br>to responses evoked by AVP, which acts via a guanine nucleo-<br>tide-binding protein-linked signaling pathway, but include re-<br>sponses stimulated by platelet-der also demonstrate that handline actions are not specific solely<br>to responses evoked by AVP, which acts via a guanine nucleo-<br>tide-binding protein-linked signaling pathway, but include re-<br>sponses stimulated by platelet-der to responses evoked by Avr, which acts via a guarime nucleotide-binding protein-linked signaling pathway, but include responses stimulated by platelet-derived growth factor, an agonist that elevates  $[Ca^{2+}]_i$  via recepto ude-unraing protein-inned signaling patriway, but inclusionses stimulated by platelet-derived growth factor, an a<br>that elevates  $[Ca^{2+}]$ , via receptor-latent tyrosine kinase a<br>The current results demonstrate that, in vasc

Volatile anesthetics such as halothane not only abolish con-Volatile anesthetics such as halothane not only abolish consciousness but also depress the circulation, in part by a direct action on arteries and arterioles (1, 2). Despite important Volatile anesthetics such as halothane not only abolish consciousness but also depress the circulation, in part by a direct action on arteries and arterioles (1, 2). Despite important clinical consequences, which include b Volatile anesthetics such as halothane not only abolish consciousness but also depress the circulation, in part by a direct action on arteries and arterioles (1, 2). Despite important clinical consequences, which include b Volatile anesthetics such as halothane not only abolish cosciousness but also depress the circulation, in part by a dire<br>action on arteries and arterioles (1, 2). Despite importa<br>clinical consequences, which include both d sciousness but also depress the circulation, in part by a direct ion action on arteries and arterioles (1, 2). Despite important althe clinical consequences, which include both decreased arterial halo blood pressure and in action on arteries and arterioles (1, 2). Despite important<br>clinical consequences, which include both decreased arterial<br>blood pressure and increased blood flow to vital organs, includ-<br>ing the heart (2) and brain (3), lit nical consequences, which include both decreased arterial<br>ood pressure and increased blood flow to vital organs, includ-<br>g the heart (2) and brain (3), little is known concerning the<br>echanisms of the vascular smooth muscle blood pressure and increased blood flow to vital organs, includ-<br>ing the heart (2) and brain (3), little is known concerning the<br>oper-<br>mechanisms of the vascular smooth muscle effects of halothane.<br>Theories of general ane

ing the heart (2) and brain (3), little is known concerning the mechanisms of the vascular smooth muscle effects of halothane<br>Theories of general anesthesia suggest that lipid-soluble volatile anesthetics have actions pred mechanisms of the vascular smooth muscle effects of halothane.<br>Theories of general anesthesia suggest that lipid-soluble vol-<br>atile anesthetics have actions predominantly at cell surface and<br>intracellular membranes, rather Theories of general anesthesia suggest that lipid-soluble vol-<br>atile anesthetics have actions predominantly at cell surface and  $Ca^{2}$ <br>intracellular membranes, rather than at sites within the cyto-<br>plasm  $(3, 4)$ . Althoug atile anesthetics have actions predominantly at cell surface and<br>intracellular membranes, rather than at sites within the cyto-<br>plasm (3, 4). Although halothane has been suggested to disorder<br>membrane bulk lipid bilayers, meracenuar memoranes, rather than at sites within the cyto-<br>plasm (3, 4). Although halothane has been suggested to disorder<br>in membrane bulk lipid bilayers, current opinion favors more<br>precise sites of action, with protein and hydrophobic pockets on sensitive proteins within membranes being possible targets (4, 5). Consequently, anesthetics<br>This work was supported by National Institutes of Health Grant HL 38668.

may alter the functions of regulatory proteins, enzymes, and<br>ion channels and interfere with cell signaling (5). Evidence, may alter the functions of regulatory proteins, enzymes, and<br>ion channels and interfere with cell signaling (5). Evidence,<br>although limited, exists to support this prediction. In neurons, may alter the functions of regulatory proteins, enzymes, and<br>ion channels and interfere with cell signaling (5). Evidence,<br>although limited, exists to support this prediction. In neurons,<br>halothane alters conductance thro may alter the functions of regulatory proteins, enzymes, an<br>ion channels and interfere with cell signaling (5). Evidence<br>although limited, exists to support this prediction. In neuron<br>halothane alters conductance through may alter the functions of regulatory proteins, enzymes, a<br>ion channels and interfere with cell signaling (5). Eviden<br>although limited, exists to support this prediction. In neuro<br>halothane alters conductance through  $Ca^{2$ ion channels and interfere with cell signaling (5). Evidence, although limited, exists to support this prediction. In neurons, halothane alters conductance through  $Ca^{2+}$ -sensitive K<sup>+</sup> channels (6) and inhibits increase although limited, exists to support this prediction. In neurons,<br>halothane alters conductance through  $Ca^{2+}$ -sensitive K<sup>+</sup> chan-<br>nels (6) and inhibits increases in  $[Ca^{2+}]_i$  due to receptor-<br>operated and voltage-gated halothane alters conductance through  $\hat{Ca}^{2+}$ -sensitive K<sup>+</sup> chan-<br>nels (6) and inhibits increases in  $[Ca^{2+}]_i$  due to receptor-<br>operated and voltage-gated  $Ca^{2+}$  influx (7). In cardiac myocytes,<br>halothane may alter nels (6) and inhibits increases in  $[Ca^{2+}]_i$  due to receptor-<br>operated and voltage-gated  $Ca^{2+}$  influx (7). In cardiac myocytes,<br>halothane may alter sarcolemmal muscarinic receptor G protein<br>function (8), inhibit voltag operated and voltage-gated  $Ca^{2+}$  influx (7). In cardiac myocytes,<br>halothane may alter sarcolemmal muscarinic receptor G protein<br>function (8), inhibit voltage-gated  $Ca^{2+}$  influx (9), and impair<br> $Ca^{2+}$  storage in the s halothane n<br>function (8)<br>Ca<sup>2+</sup> storag<br>the effects o<br>ill defined.<br>In the cu nction (8), inhibit voltage-gated Ca<sup>2+</sup> influx (9), and impair  $a^{2+}$  storage in the sarcoplasmic reticulum (10). In contrast, e effects of halothane on vascular smooth muscle cells remain defined.<br>In the current experi  $Ca^{2+}$  storage in the sarcoplasmic reticulum (10). In contrast,<br>the effects of halothane on vascular smooth muscle cells remain<br>ill defined.<br>In the current experiments, halothane was investigated for<br>its ability to inhib

the effects of halothane on vascular smooth muscle cells remain<br>ill defined.<br>In the current experiments, halothane was investigated for<br>its ability to inhibit inositol phosphate and Ca<sup>2+</sup> responses<br>stimulated by AVP. Many In defined.<br>In the current experiments, halothane was investigated fo<br>its ability to inhibit inositol phosphate and  $Ca^{2+}$  response<br>stimulated by AVP. Many vasoactive hormones, includin<br>AVP, which has an important role i In the current experiments, halothane was investigated for<br>its ability to inhibit inositol phosphate and  $Ca^{2+}$  responses<br>stimulated by AVP. Many vasoactive hormones, including<br>AVP, which has an important role in regulat stimulated by AVP. Many vasoactive hormones, including AVP, which has an important role in regulating peripheral vascular resistance (11), interact with receptors linked via G proteins to phosphoinositide-specific phosphol

vascular resistance (11), interact with receptors linked via G<br>This work was supported by National Institutes of Health Grant HL 38668. proteins to phosphoinositide-specific phospholipase C (12).<br>ABBREVIATIONS: G protein, **ABBREVIATIONS:** G protein, guanine nucleotide-binding protein; AVP, arginine vasopressin; PDGF, platelet-derived growth factor; [Ca<sup>2+</sup>],<br>intracellular free Ca<sup>2+</sup> concentration; EGTA, ethylene glycol bis(*j)*-aminoethyl intracellular free Ca<sup>2+</sup> concentration; EGTA, ethylene glycol bis(*β*-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; Ins(1,4,5)P<sub>3</sub>, inositol 1,4,5-trisphos-<br>phate; HEPES, 4-(2-hydroxyethyl)-l-piperazineethanesulfonic ac

This enzyme hydrolyzes membrane-bound phosphatidylinositol  $4.5$ -bisphosphate to release Ins $(1.4.5)P_3$  and diacylglycerol (13). H<br>This enzyme hydrolyzes membrane-bound phosphatidylinositol<br>4,5-bisphosphate to release Ins(1,4,5)P<sub>3</sub> and diacylglycerol (13).<br>Whereas diacylglycerol regulates cell activity via protein kinase Half This enzyme hydrolyzes membrane-bound phosphatidylinositol<br>4,5-bisphosphate to release Ins(1,4,5)P<sub>3</sub> and diacylglycerol (13).<br>Whereas diacylglycerol regulates cell activity via protein kinase<br>C, Ins(1,4,5)IP<sub>3</sub> inter This enzyme hydrolyzes membrane-bound phosphatidylinositol A4,5-bisphosphate to release  $\text{Ins}(1,4,5)\text{P}_3$  and diacylglycerol (13). pa<br>Whereas diacylglycerol regulates cell activity via protein kinase de<br>C, Ins(1,4,5)IP<sub>3</sub> I insenzyme nydrotyzes memorane-bound phosphatidy<br>infosion 4,5-bisphosphate to release Ins $(1,4,5)P_3$  and diacylglycerol (13).<br>Whereas diacylglycerol regulates cell activity via protein kinase<br>C, Ins $(1,4,5)IP_3$  interact Whereas diacylglycerol regulates cell activity via protein kinase C, Ins(1,4,5)IP<sub>3</sub> interacts with receptors on intracellular storage sites to release  $Ca^{2+}$  from portions of these stores. Pressor hormones, including AV ces to release Ca<sup>2+</sup> from portions of these stores. Pressor<br>
specifically, in addition promote extracellar Ca<sup>2+</sup> entry into cells via channels in cell surface mem-<br>
anes (15).<br>
Specifically, the current study sought fir hormones, including AVP (14), in addition promote extracel-<br>lular Ca<sup>2+</sup> entry into cells via channels in cell surface mem-<br>branes (15).<br>Specifically, the current study sought first to determine<br>whether halothane (a) alte

lular Ca<sup>2+</sup> entry into cells via channels in cell surface mem-<br>branes (15).<br>Specifically, the current study sought first to determine<br>whether halothane (a) altered resting  $[Ca^{2+}]$ ; in quiescent cells,<br>(b) attenuated inc branes (15).<br>
Specifically, the current study sought first to determine<br>
whether halothane (a) altered resting  $[Ca^{2+}]$ ; in quiescent cells,<br>
im<br>
(b) attenuated increases in  $[Ca^{2+}]$ ; evoked by AVP, (c) decreased<br>
but rel Specifically, the current study sought first to determine<br>whether halothane (a) altered resting  $[Ca^{2+}]_i$  in quiescent cells,<br>(b) attenuated increases in  $[Ca^{2+}]_i$  evoked by AVP, (c) decreased<br>release of  $Ca^{2+}$  from in whether halothane (a) altered resting  $|Ca^{2+}|\right|$ ; in quiescent cells, ime<br>
(b) attenuated increases in  $[Ca^{2+}]\right|$ ; evoked by AVP, (c) decreased but<br>
release of  $Ca^{2+}$  from internal stores, (d) attenuated  $Ca^{2+}$  influx (b) attenuated increases in  $[Ca^{2+}]_i$  evoked by AVP, (c) decreased bu<br>release of  $Ca^{2+}$  from internal stores, (d) attenuated  $Ca^{2+}$  influx tio<br>via cell surface membranes, and (e) inhibited AVP-induced<br>inositol phosphat release of Ca<sup>2+</sup> from internal stores, (d) attenuated Ca<sup>2+</sup> influ<br>via cell surface membranes, and (e) inhibited AVP-induce<br>inositol phosphate formation. Secondly, because halothane<br>believed to decrease Ca<sup>2+</sup> availabili via cell surface membranes, and (e) inhibited AVP-induced<br>inositol phosphate formation. Secondly, because halothane is<br>believed to decrease  $Ca^{2+}$  availability for contraction in cardiac<br>muscle, in part by depleting  $Ca^{2$ smooth phosphate formation. Secondly, because halo that is<br>believed to decrease  $Ca^{2+}$  availability for contraction in cardiac<br>muscle, in part by depleting  $Ca^{2+}$  from the sarcoplasmic retic-<br>ulum (16), the effects of h believed to decrease  $Ca^{2+}$  availability for contraction in cardiac muscle, in part by depleting  $Ca^{2+}$  from the sarcoplasmic retic-<br>ulum (16), the effects of halothane on  $Ca^{2+}$  storage in vascular<br>smooth muscle cells muscle, in part by depleting  $Ca^{2+}$  from the sarcoplasmic retic-<br>ulum (16), the effects of halothane on  $Ca^{2+}$  storage in vascular<br>smooth muscle cells were addressed by stimulating cells with<br>thapsigargin. This probe di ulum (16), the effects of halothane on  $Ca^{2+}$  storage in vascular smooth muscle cells were addressed by stimulating cells with thapsigargin. This probe discharges  $Ca^{2+}$  from  $Ins(1,4,5)P_3$ -sensitive intracellular store smooth muscle cells were addressed by stimulating cells with<br>thapsigargin. This probe discharges  $Ca^{2+}$  from  $Ins(1,4,5)P_3$ -<br>sensitive intracellular stores (17), with the magnitude of the<br> $[Ca^{2+}]$ ; response representing t sensitive intracellular stores (17), with the magnitude of the [Ca<sup>2+</sup>]; response representing the amount of releasable Ca<sup>2+</sup> is present in the stores. Finally, in order to exclude a halothane effect specific to AVP, whi  $[Ca<sup>2+</sup>]$ ; response representing the amount of releasable Ca<sup>-1</sup><br>present in the stores. Finally, in order to exclude a halothane<br>effect specific to AVP, which elevates  $[Ca<sup>2+</sup>]$ ; via a G protein-<br>linked signaling syst present in the stores. Finany, in other to exclude a harothane effect specific to AVP, which elevates  $[Ca^{2+}]_i$  via a G protein-<br>linked signaling system (12), experiments were also performed<br>by stimulating the cells with linked signaling system (12), experiments were also performed<br>by stimulating the cells with PDGF. This agonist, in contrast<br>to AVP, evokes an increase in  $[Ca^{2+}]$ ; via receptor dimerization,<br>autophosphorylation, and uncov by stimulating the complete to AVP, evokes an inductional state of the state of the state of the control pholipase C (18).<br>Experiments were AVP, evokes an increase in  $[Ca^{2+}]$ ; via receptor dimerization, and tophosphorylation, and uncovering of latent receptor tyro-<br>ne kinase activity, with possible direct stimulation of phos-<br>lase C (18).<br>Experiments were pe

autophosphorylation, and uncovering of latent receptor tyrosine kinase activity, with possible direct stimulation of phospholipase C (18).<br>
Experiments were performed using a vascular smooth muscle<br>
cell line derived origi sine kinase activity, with possible direct stimulation of phos-<br>pholipase C (18).<br>
Experiments were performed using a vascular smooth muscle<br>
cell line derived originally from rat aorta (19), which exhibits<br>
well character Experiments were performed using a vascular smooth muscle cell line derived originally from rat aorta  $(19)$ , which exhibits well characterized responses to vasoactive hormones including  $AVP (20)$ . The cells were preincuba well characterized responses to vasoactive hormones including AVP (20). The cells were preincubated with halothane concentrations corresponding to those used in clinical practice, i.e., 0.5-2.0% in the gas phase. trations corresponding to those used in clinical practice, i.e., 0.5–2.0% in the gas phase.<br>**Experimental Procedures** 

**Experimental Procedures**<br>**Experimental Procedures**<br>**Materials and chemicals.** AVP and thapsigargin were obtained<br>from Calbiochem (San Diego, CA). Human recombinant PDGF  $\beta$  chain<br>homodimer was purchased from Bachem Inc. **Experimental Procedures**<br>Materials and chemicals. AVP and thapsigargin were obtaine<br>from Calbiochem (San Diego, CA). Human recombinant PDGF  $\beta$  chai<br>homodimer was purchased from Bachem Inc. (Torrance, CA). Halo<br>thane wa **Materials and chemicals**. AVP and thapsigargin were obtained from Calbiochem (San Diego, CA). Human recombinant PDGF  $\beta$  chain homodimer was purchased from Bachem Inc. (Torrance, CA). Halo-<br>thane was purchased from Ayer from Calbiochem (San Diego, CA). Human recombinant PDGF  $\beta$  chain<br>homodimer was purchased from Bachem Inc. (Torrance, CA). Halo-<br>thane was purchased from Ayerst Laboratories (New York, NY). Indo-<br>1 pentaacetoxymethyl est homodimer was purchased from Bachem Inc. (Torrance, CA). Halo-<br>
thane was purchased from Ayerst Laboratories (New York, NY). Indo-<br>
1 pentaacetoxymethyl ester was purchased from Molecular Probes<br>
(Eugene, OR) and digitoni 1 pentaacetoxymethyl ester was purchased from Molecular Probes (Eugene, OR) and digitonin from the Sigma Chemical Company (St. Louis, MO).  $myo$ <sup>[3</sup>H]Inositol was purchased from Amersham Corp. (Arlington Heights, IL). DMEM, (Eugene, OR) and digitonin from the Sigma Chemical Company (St. Louis, MO).  $myo$ -[<sup>3</sup>H]Inositol was purchased from Amersham Corp. (Arlington Heights, IL). DMEM, MEM (no Ca<sup>2+</sup>), inositol-free medium (medium 199), HEPES, a (Arlington Heights, IL). DMEM, MEM (no Ca<sup>2+</sup>), inositol-free medium (medium 199), HEPES, and trypsin were obtained from GIBCO. Fetal bovine serum was acquired from Hyclone (Logan, VT).<br>Cell culture. The A7r5 cell line was

(medium 199), HEPES, and trypsin were obtained from GIBCO. Fetal<br>bovine serum was acquired from Hyclone (Logan, VT).<br>**Cell culture**. The A7r5 cell line was obtained from the American<br>Type Culture Collection (Rockville, MD bovine serum was acquired from Hyclone (Logan, VT).<br>
Cell culture. The A7r5 cell line was obtained from the American<br>
Type Culture Collection (Rockville, MD), (ATCC number CRL 1444).<br>
The cells were grown in flasks contain The cens were grown in hasks containing DNEN1 with 10% letained<br>bovine serum, 100 units/ml penicillin, and 100  $\mu$ g/ml streptomycin, at<br>37° in a humidified 95% air/5%  $CO_2$  atmosphere. Experiments were and ther<br>performed 37° in a humidified 95% air/5%  $CO_2$  atmosphere. Experiments were performed with cells passaged once every 5-7 days for not more than 12 weeks.<br> **Estimation of [Ca<sup>2+</sup>]**<sub>1</sub>. The cultured cells were incubated with 4  $\mu$ M

37\* (20) (indo-1/acetoxymethyl ester is hydrolyzed in the cells were incubated with  $4 \mu$ M<br>  $\mu$ indo-1/acetoxymethyl ester plus 0.1% pluronic acid for 35-40 min at  $37^{\circ}$  (20) (indo-1/acetoxymethyl ester is hydrolyzed i saline, the cells were gently dissociated with  $\frac{1}{2}$  plurid and  $-1$ /acetoxymethyl ester is hydrolyzed in the cell to the incell-impermeant acidic form). After washing with phosphate-buffered saline, the cells were ge served in the containing 25 mM Hernandical sum containing with phosphate-buffered saline, the cells were gently dissociated with  $0.05\%$  trypsin for 5 min, representing 25 mM HEPES. The cell suspension was centrifuged, a saline, the cells were gently dissociated with 0.05% trypsin for 5 min, centrifuged, washed, and resuspended in DMEM plus 10% fetal bovine serum containing 25 mM HEPES. The cell suspension was centrifuged, and the concentr

**Iothane Inhibits Inositol Phosphate and [Ca<sup>2+</sup>], Responses 1007**<br>Aeration with halothane was performed before addition of the cells, by<br>passage of 95%  $O_2/5\%$  CO<sub>2</sub> gas mixture containing the anesthetic, **Iothane Inhibits Inositol Phosphate and**  $[Ca^{2+}]$  **Responses** 1007<br>Aeration with halothane was performed before addition of the cells, by<br>passage of 95%  $O_2/5\%$   $CO_2$  gas mixture containing the anesthetic<br>delivered by a **lothane inhibits inositol Phosphate and [Ca<sup>2+</sup>], Responses 1007**<br>Aeration with halothane was performed before addition of the cells, by<br>passage of  $95\%$  O<sub>2</sub>/5% CO<sub>2</sub> gas mixture containing the anesthetic,<br>delivered by Aeration with halothane was performed before addition of the cells, passage of  $95\%$  O<sub>2</sub>/5% CO<sub>2</sub> gas mixture containing the anesthe delivered by a calibrated vaporizer at 150 ml/min for 20 min, throu 25 ml of medium at Aeration with halothane was performed before addition of the cells, by passage of  $95\%$   $O_2/5\%$   $CO_2$  gas mixture containing the anesthetic, delivered by a calibrated vaporizer at  $150$  ml/min for  $20$  min, through  $25$ delivered by a calibrated vaporizer at 150 ml/min for 20 min, through 25 ml of medium at 37°, in a semiclosed container. Halothane concentration in the aerating gas was adjusted using an anesthetic analyzer (Siemens 120, S 25 ml of medium at 37°, in a semiclosed container. Halothane concentration in the aerating gas was adjusted using an anesthetic analyzer (Siemens 120, Solna, Sweden), which itself had been calibrated using commercially ava tration in the aerating gas was adjusted using an anesthetic analyzer (Siemens 120, Solna, Sweden), which itself had been calibrated using commercially available gas mixtures of known concentration (Scott Medical, Plumstea commercially available gas mixtures of known concentration (Sc<br>Medical, Plumstead PA) and by using a mass spectrometer (Perk<br>Elmer 1100, Pomona, CA). The medium was aspirated into airtiglass syringes to prevent evaporation Medical, Plumstead PA) and by using a mass spectrometer (Perkins Elmer 1100, Pomona, CA). The medium was aspirated into airtight glass syringes to prevent evaporation of the anesthetic. Control experiments were performed u glass syringes to prevent evaporation of the anesthetic. Control experiments were performed using medium treated in an identical manner but in the absence of the anesthetic. Appropriate halothane concentrations in the medi glass syringes to prevent evaporation of the anesthetic. Control experiments were performed using medium treated in an identical manner but in the absence of the anesthetic. Appropriate halothane concentrations in the med iments were performed using medium treated in an identical manner<br>but in the absence of the anesthetic. Appropriate halothane concentra-<br>tions in the medium were confirmed by extracting the anesthetic from<br> $400 \mu$  of medi but in the absence of the anesthetic. Appropriate halothane concentra-<br>tions in the medium were confirmed by extracting the anesthetic from<br>400  $\mu$ l of medium into 2 ml of hexane and analyzing for halothane<br>content by ga tions in the medium were confirmed by extracting the anesthetic from  $400 \mu$  of medium into 2 ml of hexane and analyzing for halothane content by gas chromatography with electron capture (Hewlett Packard 5880A), in argon/ content by gas chromatography with electron capture (Hewlett Packard 5880A), in argon/methane carrier gas. During the period of the experiment, a close correlation was achieved between desired anesthetic concentrations and 5880A), in argon/methane carrier gas. During the period of the experiment, a close correlation was achieved between desired anesthetic concentrations and actual concentrations measured in the culture medium. (Halothane lev concentrations and actual concentrations measured in the culture<br>medium. (Halothane levels in the medium were expressed as gas phase<br>concentrations predicted using known partition coefficients for distri-<br>bution between aq medium. (Halothane levels in the medium were expressed as gas phase<br>concentrations predicted using known partition coefficients for distri-<br>bution between aqueous and gas phases.) Anesthetic concentrations<br>in the aerating bution between aqueous and gas phases.) Anesthetic concentrations in the aerating gas of 0.5, 1.25, 1.5, and 2.0% resulted in concentrations in the culture medium that corresponded to 0.69  $\pm$  0.04, 1.09  $\pm$  0.07, 1.62

0.5–2.0% in the gas phase.<br> **Experimental Procedures**<br> **Experimental Procedures**<br>
Materials and chemicals. AVP and thapsigargin were obtained<br>
Materials and chemicals. AVP and thapsigargin were obtained<br>
Materials and che the aerating gas of 0.5, 1.25, 1.5, and 2.0% resulted in concentrations<br>in the culture medium that corresponded to 0.69  $\pm$  0.04, 1.09  $\pm$  0.07,<br>1.62  $\pm$  0.05, and 2.08  $\pm$  0.09%, respectively.<br>The cells were preincub in the culture medium that corresponded to  $0.69 \pm 0.04$ ,  $1.09 \pm 0.07$ ,  $1.62 \pm 0.05$ , and  $2.08 \pm 0.09\%$ , respectively.<br>The cells were preincubated at room temperature in medium with or without halothane for 10 min, to 1.62  $\pm$  0.05, and 2.08  $\pm$  0.09%, respectively.<br>The cells were preincubated at room temperature in medium with<br>or without halothane for 10 min, to allow anesthetic uptake and<br>equilibration. Apparent  $[Ca^{2+}]_i$  was meas The cells were preincubated at room temperature in medium with<br>or without halothane for 10 min, to allow anesthetic uptake and<br>equilibration. Apparent  $[Ca^{2+}]_i$  was measured by passing cells through<br>a flow cytometer (20) or without halothane for 10 min, to allow anesthetic uptake and<br>equilibration. Apparent  $[Ca^{2+}]$ , was measured by passing cells through<br>a flow cytometer (20) (FACStar-Plus; Beckton Dickinson, Mountain<br>View, CA) (adapted f a flow cytometer (20) (FACStar-Plus; Beckton Dickinson, Mountain View, CA) (adapted for this purpose) (21), where a stream of cells (300–500 cells/sec) was excited with 351–364-nm light emitted from an argon laser. Fluores 385-395 nm, using appropriate band pass filters in front of photomul-500 cells/sec) was excited with 351-364-nm light emitted from an argon<br>laser. Fluorescence was measured simultaneously at 480-520 nm and<br>385-395 nm, using appropriate band pass filters in front of photomul-<br>tiplier tubes 385-395 nm, using appropriate band pass filters in front of photomul-<br>tiplier tubes and fluorescence ratios calculated for each cell. The cells<br>were stimulated by addition of  $10^{-9}$  M or  $10^{-7}$  M AVP,  $3.3 \times 10^{-6}$  M<br>PD tiplier tubes and fluorescence ratios calculated for each cell. The cells<br>were stimulated by addition of  $10^{-9}$  M or  $10^{-7}$  M AVP,  $3.3 \times 10^{-9}$  M<br>PDGF, or  $5 \times 10^{-5}$  M thapsigargin directly into the cell suspension,<br>w concentrations and actual concentrations measured in the culture medium. (Halothane levels in the medium were repressed as age phase.) Antention coefficients for distribution between aquosus and gas phases.) Anesthetic co cells (20) and contracts isolated blood vessels. The PDGF dose was chosen because it evokes marked  $[Ca^{2+}]_i$  responses in Swiss 3T3 cells.<br>The thapsigargin dose was chosen after pilot experiments.] In some experiments, c chosen because it evokes marked  $[Ca^{2+}]$ ; responses in Swiss 3T3 cells.<br>The thapsigargin dose was chosen after pilot experiments.] In some<br>experiments, cells were suspended in  $Ca^{2+}$ -free MEM plus 0.5 mM<br>EGTA and stimula experiments, cells were suspended in Ca<sup>2+</sup>-free MEM plus 0.5 mM EGTA and stimulated with AVP or with thapsigargin, in order to evoke increases in  $[Ca^{2+}]_i$  due to  $Ca^{2+}$  discharge from intracellular stores. In other ex EGTA and stimulated with AVP or with thapsigargin, in order to evoke<br>increases in  $[Ca^{2+}]_i$  due to  $Ca^{2+}$  discharge from intracellular stores. In<br>other experiments,  $Ca^{2+}$  influx from extracellular sources was investi-EGTA and stimulated with AVP or with thapsigargin, in order to evoke<br>increases in  $[Ca^{2+}]_i$  due to  $Ca^{2+}$  discharge from intracellular stores. In<br>other experiments,  $Ca^{2+}$  influx from extracellular sources was investiincreases in  $[Ca^{2+}]_i$  due to  $Ca^{2+}$  discharge from intracellular stores. In other experiments,  $Ca^{2+}$  influx from extracellular sources was investigated by stimulation of cells with  $10^{-7}$  M AVP while they were suspe gated by stimulation of cells with  $10^{-7}$  M AVP while they were suspended in Ca<sup>2+</sup>-free MEM plus 0.5 mM EGTA. After completion of the initial  $[Ca^{2+}]_i$  transient, 5 mM CaCl<sub>2</sub> was added in the continued presence of AVP 15). nded in  $Ca^{2+}$ -free MEM plus 0.5 mM EGTA. After completion of the<br>itial  $[Ca^{2+}]_i$  transient, 5 mM CaCl<sub>2</sub> was added in the continued<br>esence of AVP, resulting in a second, sustained,  $[Ca^{2+}]_i$  response (14,<br>).<br>The immed initial  $[Ca^{2+}]_i$  transient, 5 mM CaCl<sub>2</sub> was added in the continued<br>presence of AVP, resulting in a second, sustained,  $[Ca^{2+}]_i$  response (14,<br>15).<br>The immediate effects of acute addition of halothane solution on<br> $[Ca^{2$ 

presence of AVP, resulting in a second, sustained,  $[Ca^{2+}]$ , response (14<br>15).<br>The immediate effects of acute addition of halothane solution or<br> $[Ca^{2+}]$ , in quiescent unstimulated cells were assessed by addition of<br>haloth The immediate effects of acute addition of halothane solution on  $[Ca^{2+}]_i$  in quiescent unstimulated cells were assessed by addition of halothane dissolved in culture medium directly into the suspension, without interrup halothane dissolved in culture medium directly into the suspension,<br>without interruption of the flow of the cells, to obtain levels correspond-<br>ing to 1% and 2% gas phase concentrations. (The halothane solution<br>was prepar ing to 1% and 2% gas phase concentrations. (The halothane solution was prepared by dissolving 25  $\mu$ l of liquid halothane in 975  $\mu$ l of DMSO and then diluting this solution further in predetermined volumes of medium. D was prepared by dissolving 25  $\mu$ l of liquid halothane in 975  $\mu$ l of DMSO and then diluting this solution further in predetermined volumes of medium. DMSO at this concentration had no independent effect on  $[Ca^{2+}]_i$ .) and then diluting this solution further in predetermined volumes of medium. DMSO at this concentration had no independent effect on  $[Ca^{2+}]_i$ .) The gas phase above the cell suspension was flushed with  $O_2$ / $CO_2$  mixture medium. DMSO at this concentration had no independent  ${[Ca^{2+}]_i}$ .) The gas phase above the cell suspension was flushed  $CO_2$  mixture containing 1% or 2% halothane, in order to evaporation of the anesthetic. Appropriate  $a^{2+}$ ]..) The gas phase above the cell suspension was flushed with  $O_{2}$ , mixture containing 1% or 2% halothane, in order to preven aporation of the anesthetic. Appropriate halothane concentration the aqueous phase wer CO<sub>2</sub> mixture containing 1% or 2% halothane, in order to prever evaporation of the anesthetic. Appropriate halothane concentration the aqueous phase were confirmed using gas chromatography.<br>Absence of nonspecific halothan

evaporation of the anesthetic. Appropriate halothane concentrations<br>in the aqueous phase were confirmed using gas chromatography.<br>Absence of nonspecific halothane effects on indo-1 fluorescence,<br>reported by others (22), w in the aqueous phase were confirmed using gas chromatography.<br>Absence of nonspecific halothane effects on indo-1 fluorescence,<br>reported by others (22), was reassessed by measuring indo-1 fluores-<br>cence ratios in cells tre Absence of nonspecific halothane<br>reported by others (22), was reassesse<br>cence ratios in cells treated with 2  $\mu$ h<br>absence of the anesthetic. Peak fluore<br> $\pm$  0.01; 1.5% halothane, 0.60  $\pm$  0.01.<br>All protocols were repea ported by others (22), was reassessed by measuring indo-1 fluores-<br>nce ratios in cells treated with  $2 \mu M$  digitonin, in the presence and<br>sence of the anesthetic. Peak fluorescence ratios were controls, 0.62<br>0.01; 1.5% ha

**1008** Sill of al.<br>minimum of three separate periods. Each individual experiment was TABLE 1<br>accompanied by a control. Indo-1 fluorescence ratios were converted immedia **1008 Sill of al.**<br>minimum of three separate periods. Each individual experiment was<br>accompanied by a control. Indo-1 fluorescence ratios were converted<br>to  $[Ca^{2+}]_i$  values by exposing cells acutely permeabalized with **1008 Sill of al.**<br>
minimum of three separate periods. Each individual experiment was TABLE 1<br>
accompanied by a control. Indo-1 fluorescence ratios were converted immedia<br>
to  $[Ca^{2+}]$ , values by exposing cells acutely accompanied by a control. Indo-1 fluorescence ratios were converted<br>to  $[Ca^{2+}]_i$  values by exposing cells acutely permeabalized with 10  $\mu$ M<br>digitonin to different concentrations of extracellular  $Ca^{2+}$  and then,<br>using to  $[Ca^{2+}]_i$  values by exposing cells acutely permeabalized with 10  $\mu$ M digitonin to different concentrations of extracellular  $Ca^{2+}$  and then, using an indo  $K_D$  of 250 nM and measuring and calculating the ratio of f digitonin to different concentrations of extracellular  $Ca^{2+}$  and then, applying an indo  $K_D$  of 250 nM and measuring and calculating the ratio of from fluorescence of  $Ca^{2+}$ -free indicator and  $Ca^{2+}$ -bound indicator, many an material fluorescence of  $Ca^{2+}$ -<br>(2.5), maximum fluor<br>ratio (0.1), solving the used by others (21).<br>Measurement of i mg an into  $Kp$  or 200 lim and measuring and calculating the ratio of orescence of  $Ca^{2+}$ -free indicator and  $Ca^{2+}$ -bound indicator,  $S_f/S_b$  in cell (5), maximum fluorescence ratio (1.1), and minimum fluorescence the cel

(2.5), maximum fluorescence ratio (1.1), and minimum fluorescence<br>ratio (0.1), solving the equation described by Grynkiewicz (23) and<br>used by others (21).<br>**Measurement of inositol phosphates**. Cells were grown in 35-mm<br>cu ratio (0.1), solving the equation described by Grynkiewicz (23) and<br>used by others (21).<br>**Measurement of inositol phosphates**. Cells were grown in 35-mm<br>culture dishes for 5-7 days, to reach confluence, and were labeled f used by others (21).<br> **Measurement of inositol phosphates**. Cells were grown in 35-mm<br>
culture dishes for 5-7 days, to reach confluence, and were labeled for<br>
21 hr with  $myo$ -[<sup>3</sup>H]inositol (18.2 Ci/mmol in inositol-free **Measurement of inositol phosphates.** Cells were grown in 35-mm culture dishes for 5-7 days, to reach confluence, and were labeled for 21 hr with  $myo$ -[<sup>3</sup>H]inositol (18.2 Ci/mmol in inositol-free medium without fetal bov 21 hr with  $myo$ -[<sup>3</sup>H]inositol (18.2 Ci/mmol in inositol-free medium without fetal bovine serum, buffered with 10 mM HEPES. They were washed with balanced salt solution containing 3.0 mM HEPES, pH 7.4, and then harvested, without fetal bovine serum, buffered with 10 mM HEPES. They were washed with balanced salt solution containing 3.0 mM HEPES, pH 7.4,

washed with balanced salt solution containing 3.0 mM HEPES, pH 7.4,<br>and then harvested, using  $0.05\%$  trypsin for 10 min, and centrifuged.<br>Experiments were performed after addition of the cell suspension to<br>balanced salt and then harvested, using  $0.05\%$  trypsin for 10 min, and centrifuged.<br>Experiments were performed after addition of the cell suspension to<br>balanced salt solution, with or without halothane, in a closed container<br>at 37°. Experiments were performed after addition of the cell suspension to<br>balanced salt solution, with or without halothane, in a closed container<br>at 37°. Addition of halothane was performed before addition of the<br>cells, by pre balanced salt solution, with or without halothane, in a closed container<br>at 37°. Addition of halothane was performed before addition of the<br>cells, by preparing halothane in DMSO (described above) and balanced<br>salt solutio at 37°. Addition of halothane was performed before addition of the cells, by preparing halothane in DMSO (described above) and balanced meal solution. After preincubation for 10 min and addition of 20 mM LiCl to prevent in solution. After preincubation for 10 min and addition of 20 mM lto prevent inositol phosphate breakdown, 0.5 ml of cell suspension transferred to test tubes in a water bath at 37° in a semiclosed cham Halothane loss by eva to prevent inositol phosphate breakdown, 0.5 ml of cell suspension was<br>transferred to test tubes in a water bath at 37° in a semiclosed chamber.<br>Halothane loss by evaporation was prevented by continuously passing<br>air-conta Flalothane loss by evaporation was prevented by continuously parain-containing halothane through the chamber. Anesthetic concentrations were monitored and adjusted as described above. Anest concentrations of 1% and 2% in air-containing halothane through the chamber. Anesthetic concentra-<br>tions were monitored and adjusted as described above. Anesthetic<br>concentrations of 1% and 2% in the aerating gas resulted in concentra-<br>tions in the bala respectively. tions were monitored and adjusted as described above. Anesthetic EGTA v<br>
concentrations of 1% and 2% in the aerating gas resulted in concentra-<br>
tions in the balanced salt solution of 1.01  $\pm$  0.02% and 2.16  $\pm$  0.03%,

respectively.<br>
Inositol phosphate formation was stimulated by addition of  $10^{-7}$  M<br>
AVP to the test tubes, and the effect was terminated at predetermined<br>
times by lysing of the cells with 120  $\mu$ l of 0.22 N HCl and 900 Inositol phosphate formation was stimulated by addition of  $10^{-7}$  M<br>AVP to the test tubes, and the effect was terminated at predetermined<br>times by lysing of the cells with 120  $\mu$ l of 0.22 N HCl and 900  $\mu$ l of<br>chlorof times by lysing of the cells with 120  $\mu$ l of 0.22 N HCl and 900  $\mu$ l chloroform/methanol (1:2). Chloroform/water (1:1) (1.8 ml) was add<br>in order to extract inositol phosphates into the aqueous phase a<br>after centrifugat chloroform/methanol (1:2). Chloroform/water (1:1) (1.8 ml) was added<br>in order to extract inositol phosphates into the aqueous phase and,<br>after centrifugation, the aqueous phase was removed, diluted 8-fold in<br>water, and app after centrifugation, the aqueous phase was removed, diluted 8-fold in increase in  $[Ca^{2+}]$ , followed by a rapid and more complete water, and applied to anion exchange columns containing approximately the metally 0.5 ml o after centrifugation, the aqueous phase was removed, diluted 8-fold in water, and applied to anion exchange columns containing approximately 0.5 ml of mixed bed resin (Dowex AG 1-×8, 200–400 mesh, in the formate form; Biowater, and applied to anion exchange columns containing approximately 0.5 ml of mixed bed resin (Dowex AG 1-×8, 200–400 mesh, in in ithe formate form; Bio-Rad, Irvine, CA). The columns were washed and with 20 ml of 60 mM s the formate form; Bio-Rad, Irvine, CA). The columns were washed<br>with 20 ml of 60 mM sodium formate, 5 mM sodium borate, before total<br>inositol phosphates were eluted with 5 ml of 1.2 M ammonium formate,<br>100 mM formic acid. inositol phosphates were eluted with 5 ml of 1.2 M ammonium formate,<br>100 mM formic acid. Radioactivity in the eluate was determined by<br>liquid scintillation counting (Aquassure scintillation solution; DuPont,<br>and Beckman L

liquid scintillation counting (Aquassure scintillation solution; DuPont,<br>and Beckman LS 5000 TD counter).<br>**Data analysis.** Results are expressed as mean  $\pm$  standard error.<br>Changes in indo-1 fluorescence signal ratio are and Beckman LS 5000 TD counter).<br> **Data analysis.** Results are expressed as mean  $\pm$  standard error.<br>
Changes in indo-1 fluorescence signal ratio are expressed as apparent<br>  $[Ca^{2+}]_i$ . Integrated areas under time-respons Changes in indo-1 fluorescence signal ratio are expressed as apparent  $[Ca^{2+}]$ . Integrated areas under time-response curves were obtained using apparent  $[Ca^{2+}]$ , values. Inositol phosphate levels are expressed as emissio [Ca<sup>2+</sup>]<sub>i</sub>. Integrated areas under time-response curves were of using apparent  $[Ca^{2+}]_i$  values. Inositol phosphate levels are ex as emission cpm. The *n* value represents the number of inceperiments. Data were analyzed

# **Results**

Acute effects of halothane on [Ca<sup>2+</sup>], in quiescent cells.<br>
dition of halothane on [Ca<sup>2+</sup>], in quiescent cells.<br>
dition of halothane solution directly into quiescent cell sus-Results<br>
Addition of halothane on  $[Ca^{2+}]$ , in quiescent cells.<br>
Addition of halothane solution directly into quiescent cell sus-<br>
pensions, to achieve anesthetic concentrations corresponding<br>
to 1% and 2%, produced immedi Acute effects of halothane on  $[Ca^{2+}]_1$  in quiescent cells.<br>Addition of halothane solution directly into quiescent cell sus-<br>pensions, to achieve anesthetic concentrations corresponding<br>to 1% and 2%, produced immediate b **Acute effects of halothane on**  $[Ca^{2+}]_i$  **in quiescent cells.** haddition of halothane solution directly into quiescent cell sus-<br>pensions, to achieve anesthetic concentrations corresponding Tto 1% and 2%, produced immedia Addition of halothane solution directly into quiescent cell sus-<br>pensions, to achieve anesthetic concentrations corresponding Tables 2 and 3. Inhibition of the  $[Ca^{2+}]$ , response, expressed as<br>to 1% and 2%, produced immed pensions, to achieve anesthetic concentrations corresponding<br>to 1% and 2%, produced immediate but very small and brief<br>(approximately 50 sec.) increases in  $[Ca^{2+}]_i$ . The responses<br>could barely be distinguished from the b to 1% and 2%, produced immediate but very small and brief are (approximately 50 sec.) increases in  $[Ca^{2+}]$ . The responses Fig could barely be distinguished from the background effect of shot turbulence caused by addition (approximately 50 sec.) increases in  $[Ca^{2+}]$ . The responses Fig<br>could barely be distinguished from the background effect of shc<br>turbulence caused by addition of control or anesthetic solutions. In<br>Direct addition of halo could barely be distinguished from the backgroturbulence caused by addition of control or anesth<br>Direct addition of halothane solution to cells<br>medium plus EGTA also resulted in immediate<br>small and brief increases in  $[Ca^{$ rbulence caused by addition of control or anesthetic solutions. Einerct addition of halothane solution to cells in  $Ca^{2+}$ -free in [Cadium plus EGTA also resulted in immediate but also very suspeall and brief increases in Direct addition of halothane solution to cells in  $Ca^{2+}$ -free in medium plus EGTA also resulted in immediate but also very sus small and brief increases in  $[Ca^{2+}]_i$  (Table 1). 10<sup>-</sup><br>**Effects of preincubation with halot** 

medium plus EGTA also resulted in immediate but also very s<br>small and brief increases in  $[Ca^{2+}]_i$  (Table 1).<br>**Effects of preincubation with halothane on**  $[Ca^{2+}]_i$  **in s<br>quiescent cells.** In a separate experiment, base-l

# TABLE 1<br>TABLE 1<br>Immediate effect: TABLE 1<br>Immediate effects of acute addition of halothane solution on [Ca<sup>2+</sup>],<br>in quiescent cells **TABLE 1<br>Immediate effects of act<br>in quiescent cells<br>Apparent [Ca<sup>2+</sup>], was calculate**

TABLE 1<br>I**mmediate effects of acute addition of halothane solution on [Ca<sup>2+</sup>],<br>in quiescent cells<br>Apparent [Ca<sup>2+</sup>], was calculated using the ratios of light emitted at dual wavelengths<br>from indo-1-loaded A7r5 cells image** TABLE 1<br>Immediate effects of acute addition of halothane solution on  $[Ca^{2+}]$ ,<br>in quiescent cells<br>Apparent  $[Ca^{2+}]$ , was calculated using the ratios of light emitted at dual wavelengths<br>from indo-1-loaded A775 cells image from indo-1-loaded A7r5 cells imaged using flow cytometry. Halothane dissolved<br>in cell culture medium or control solution (no halothane) was acutely injected into



Final concentration in the cell suspension.<br>
"Increases in [Ca<sup>2+</sup>] observed after injection of control solution (no halothane)<br>
may represent the effect of turbulence.<br>  ${}^{\circ} \rho < 0.01$ , in comparison with control.<br>
10 mi Thereases in [Car' j observed after injection of control solution (no halomane)<br>may represent the effect of turbulence.<br> $\degree \rho$  < 0.01, in comparison with control.<br>10 min was slightly lower than that in control untreated c  $\epsilon_p < 0.01$ , in comparison with control.<br>10 min was slightly lower than that in control untreated cells.<br>Small depressant effects of the anesthetic were observed at<br>both 1.25 and 2.0% halothane concentrations but not afte 10 min was slightly lower than that in control untreated cells.<br>Small depressant effects of the anesthetic were observed at<br>both 1.25 and 2.0% halothane concentrations but not after<br>0.5% halothane. Preincubating cells in Small depressant effects of the anesthetic were observed at both 1.25 and 2.0% halothane concentrations but not after 0.5% halothane. Preincubating cells in  $Ca^{2+}$ -free medium plus EGTA with 1.5% halothane also decreased both 1.25 and 2.0% halothane concentrations<br>0.5% halothane. Preincubating cells in  $Ca^{2+}$ -fre<br>EGTA with 1.5% halothane also decreased resti<br>bles 2 and 3). Base-line  $[Ca^{2+}]$ , varied with each<br>but the effects of halothane 5% halothane. Preincubating cells in  $Ca^{2+}$ -free medium plus GTA with 1.5% halothane also decreased resting  $[Ca^{2+}]_i$  (Ta-<br>es 2 and 3). Base-line  $[Ca^{2+}]_i$  varied with each batch of cells,<br>t the effects of preincubatio

EGTA with 1.5% halothane also decreased resting  $[Ca^{2+}]_i$  (Tables 2 and 3). Base-line  $[Ca^{2+}]_i$  varied with each batch of cells, but the effects of halothane remained consistent.<br>**Effects of preincubation with halothane** but the effects of halothane remained consistent.<br> **Effects of preincubation with halothane on increases**<br>
in  $[Ca^{2+}]_i$  evoked by AVP. Stimulation of untreated control<br>
cells with  $10^{-9}$  M AVP produced a rapid increase control cells with  $10^{-7}$  M AVP evoked a rapid and more marked **Effects of preincubation with halothane on increases**<br>in  $[Ca^{2+}]_i$  evoked by AVP. Stimulation of untreated control<br>cells with  $10^{-9}$  M AVP produced a rapid increase in  $[Ca^{2+}]_{i}$ ,<br>followed by a gradual return towards in  $[Ca^{2+}]_i$  evoked by AVP. Stimulation of untreated control<br>cells with  $10^{-9}$  M AVP produced a rapid increase in  $[Ca^{2+}]_i$ ,<br>followed by a gradual return towards base-line. Stimulation of<br>control cells with  $10^{-7}$  M A return towards base-line. Stimulation of control cells with  $10^{-7}$  M AVP evoked a rapid and more marked increase in  $[Ca^{2+}]_i$ , followed by a rapid and more complete return towards base line. Preincubation with halothane control cells with 10 'MAVP evoked a rapid and more marked<br>increase in  $[(Ca^{2+}]_i,$  followed by a rapid and more complete<br>return towards base line. Preincubation with halothane resulted<br>in inhibition of the responses evoke anesthetic on peak  $[Ca^{2+}]_i$  and  $[Ca^{2+}]_i$  at 180 sec after AVP are<br>shown in Tables 2 and 3. Tracings from typical individual<br>experiments during stimulation with  $10^{-9}$  M and  $10^{-7}$  M AVP **return towards base line. Preincubation with halothane resulted**<br>in inhibition of the responses evoked by AVP. Effects of the<br>anesthetic on peak  $[Ca^{2+}]_i$  and  $[Ca^{2+}]_i$  at 180 sec after AVP are<br>shown in Tables 2 and 3. in initiation of the responses evoked by  $AVY$ . Effects of the<br>anesthetic on peak  $[Ca^{2+}]_i$  and  $[Ca^{2+}]_i$  at 180 sec after AVP are<br>shown in Tables 2 and 3. Tracings from typical individual<br>experiments during stimulation experiments during stimulation with  $10^{-9}$  M and  $10^{-7}$  M AVP are presented in Figs. 1 and 2, respectively. Dose-dependent percentage of inhibition of the responses evoked by AVP, expressed as integrated areas under ind is presented in Figs. 1 and 2, respectively. Dose-dependent rcentage of inhibition of the responses evoked by AVP, pressed as integrated areas under individual time-response rves, are shown in Fig. 3. Effects of preincuba

percentage of inhibition of the responses evoked by AVP,<br>expressed as integrated areas under individual time-response<br>curves, are shown in Fig. 3.<br>**Effects of preincubation with halothane on increases**<br>in  $[Ca^{2+}]_i$  resul expressed as integrated areas under individual time-response<br>curves, are shown in Fig. 3.<br>**Effects of preincubation with halothane on increases**<br>in [Ca<sup>2+</sup>], **resulting from AVP-induced Ca<sup>2+</sup> release from<br>intracellular st** curves, are shown in Fig. 3.<br> **Effects of preincubation with halothane on increases**<br>
in  $[Ca^{2+}]$ , resulting from AVP-induced  $Ca^{2+}$  release from<br>
intracellular stores. The cells were suspended in  $Ca^{2+}$ -free<br>
medium pl Effects of preincubation with halothane on increases<br>in  $[Ca^{2+}]_i$  resulting from AVP-induced  $Ca^{2+}$  release from<br>intracellular stores. The cells were suspended in  $Ca^{2+}$ -free<br>medium plus EGTA, in order that increases i in  $[Ca^{2+}]_i$  resulting from AVP-induced  $Ca^{2+}$  release from<br>intracellular stores. The cells were suspended in  $Ca^{2+}$ -free<br>medium plus EGTA, in order that increases in  $[Ca^{2+}]_i$  evoked<br>by AVP would result primarily fro intracellular stores. The cells were suspended in  $Ca^{2+}$ -free<br>medium plus EGTA, in order that increases in  $[Ca^{2+}]$ , evoked<br>by AVP would result primarily from  $Ca^{2+}$  release from intra-<br>cellular stores. Stimulation with by AVP would result primarily from  $Ca^{2+}$  release from intra-<br>cellular stores. Stimulation with  $10^{-9}$  M and  $10^{-7}$  M AVP caused<br>responses that were of a lesser magnitude than those observed<br>in the presence of extracel cellular stores. Stimulation with  $10^{-9}$  M and  $10^{-7}$  M AVP caused<br>responses that were of a lesser magnitude than those observed<br>in the presence of extracellular Ca<sup>2+</sup>. Pretreatment with 1.5%<br>halothane attenuated the r responses that were of a lesser magnitude than those observed<br>in the presence of extracellular  $Ca^{2+}$ . Pretreatment with 1.5%<br>halothane attenuated the responses. The effects of halothane<br>on peak  $[Ca^{2+}]$ ; and  $[Ca^{2+}]$ ; a in the presence of extracellular  $Ca^{2+}$ . Pretreatment with 1.5% halothane attenuated the responses. The effects of halothane on peak  $[Ca^{2+}]$ <sub>i</sub> and  $[Ca^{2+}]$ <sub>i</sub> at 180 sec after AVP are shown in Tables 2 and 3. Inhibitio on peak  $[Ca^{2+}]_i$  and  $[Ca^{2+}]_i$  at 180 sec after AVP are shown in Tables 2 and 3. Inhibition of the  $[Ca^{2+}]_i$  response, expressed as areas under the integrated time response curves, is shown in Fig. 3. Recordings from

in  $[Ca^{2+}]_i$  due to AVP-induced  $Ca^{2+}$  influx. The cells were areas under the integrated time response curves, is shown in Fig. 3. Recordings from typical individual experiments are shown in Fig. 4.<br> **Effects of preincubation with halothane on increases** in [Ca<sup>2+</sup>], due to AVP-induc Fig. 3. Recordings from typical individual experiments are<br>shown in Fig. 4.<br>**Effects of preincubation with halothane on increases**<br>in  $[Ca^{2+}]_1$  due to AVP-induced  $Ca^{2+}$  influx. The cells were<br>suspended in  $Ca^{2+}$ -free **Effects of preincubation with halothane on increases** in  $[Ca^{2+}]$ , due to AVP-induced  $Ca^{2+}$  influx. The cells were suspended in  $Ca^{2+}$ -free MEM plus EGTA and stimulated with  $10^{-7}$  M AVP and then, on completion of th in  $[Ca^{2+}]$ , due to AVP-induced  $Ca^{2+}$  influx. The cells were<br>suspended in  $Ca^{2+}$ -free MEM plus EGTA and stimulated with<br> $10^{-7}$  M AVP and then, on completion of the initial  $Ca^{2+}$  tran-<br>sient, 5 mM  $Ca^{2+}$  was added i suspended in Ca<sup>21</sup>-free MEM plus EGTA and stimulated with  $10^{-7}$  M AVP and then, on completion of the initial Ca<sup>2+</sup> transient, 5 mM Ca<sup>2+</sup> was added in the continued presence of AVP. Ca<sup>2+</sup> influx occurred, resulting i

# TABLE 2<br>Effects of preincubation with halothane upon base-line [Ca<sup>2+</sup>], and upon increases in Ca<sup>2+</sup>], evoked by 10<sup>-9</sup> м AVP

The indo-1-loaded A7r5 cells were preincubated for 10 min at room temperature in culture medium, with or without halothane. Apparent [Ca<sup>2+</sup>], was obtained by Halothane Inhibits inositol Phosphate and [Ca<sup>2+</sup>], Responses 1009<br>Effects of preincubation with halothane upon base-line [Ca<sup>2+</sup>], and upon increases in Ca<sup>2+</sup>], evoked by 10<sup>-e</sup> м AVP<br>The indo-1-loaded A7r5 cells were p TABLE 2<br>Effects of preincubation with halothane upon base-line  $[Ca^{2+}]$ , and upon increases in  $Ca^{2+}$ , evoked by  $10^{-9}$  M AVP<br>The indo-1-loaded A7r5 cells were preincubated for 10 min at room temperature in culture medi



**a** Base-line [Ca<sup>2+</sup>], varied with each batch of cells. A minimum of three batches of cells were used in each experiment.<br>  $P \sim 0.01$ , in comparison with control.<br>  $P \sim 0.0001$ , in comparison with control.

**Base-line [Ca<sup>2+</sup>]**<br>  ${}^b p < 0.01$ , in com<br>  ${}^c p < 0.0001$ , in com<br>
TABLE 3<br> **Effects of preincu**  $\epsilon_p < 0.0001$ , in comparison with control.<br>
TABLE 3<br> **Effects of preincubation with halothane upon base-line [Ca<sup>2+</sup>], and upon increases in Ca<sup>2+</sup>], evoked by 10<sup>-7</sup> M AVP<br>
Beseline [Ca<sup>2+</sup>]** 



 $\frac{b}{\rho}$  < 0.01, in comparison with control.<br> $\frac{c}{\rho}$  < 0.0001, in comparison with control.



8, 0.5, 1.25, or 2.0% halothane for 10 min or incubated without the anesthetic (controls). Cell suspension was passed through a flow cytometer at 300–500 cells/sec. AVP was added without  $\overline{C}$  dual wavelengths,<br>  $\overline{C}$  dual wavelengths,<br>  $\overline{C}$  ent  $[Ca^{2+}$ ]. Results<br>
iments are shown. **Fig. 1.** Effects of preincubation with halothane<br>
on increases in [Ca<sup>2+</sup>], evoked by 10<sup>-9</sup> M AVP in<br>
cultured A7r5 vascular smooth muscle cells. The<br>
indo-1-loaded cells were incubated with either **Fig. 1.** Effects of preincubation with halothane<br>on increases in  $[Ca^{2+}]$ , evoked by  $10^{-9}$  m AVP in<br>cultured A7r5 vascular smooth muscle cells. The<br>indo-1-loaded cells were incubated with either<br>0.5, 1.25, or 2.0% halo **Fig. 1.** Effects of preincubation with halothane<br>on increases in [Ca<sup>2+</sup>], evoked by 10<sup>-9</sup> M AVP in<br>cultured A7r5 vascular smooth muscle cells. The<br>indo-1-loaded cells were incubated with either<br>0.5, 1.25, or 2.0% haloth Fig. 1. Effects of preincubation with nationale on increases in  $[Ca^{2+}]_i$  evoked by  $10^{-9}$  M AVP in cultured A7F5 vascular smooth muscle cells. The indo-1-loaded cells were incubated with either 0.5, 1.25, or 2.0% halot on increases in [Ca<sup>-1</sup>]<sub>i</sub> evoked by 10 ° M AVP in<br>cultured A7r5 vascular smooth muscle cells. The<br>indo-1-baded cells were incubated with either<br>0.5, 1.25, or 2.0% halothane for 10 min or incu-<br>bated without the anestheti cultured A/r5 vascular smooth muscle cells. The<br>indo-1-loaded cells were incubated with either<br>0.5, 1.25, or 2.0% halothane for 10 min or incu-<br>bated without the anesthetic (controls). Cell sus-<br>pension was passed through into-1-loaded cells were includated with either<br>0.5, 1.25, or 2.0% halothane for 10 min or incu-<br>bated without the anesthetic (controls). Cell sus-<br>pension was passed through a flow cytometer<br>at 300–500 cells/sec. AVP was  $0.5, 1.25, 0t \ge 0.0\%$  haloulate for 10 mm or included without the anesthetic (controls). Cell supersion was passed through a flow cytomet<br>at 300-500 cells/sec. AVP was added without<br>at 300-500 cells/sec. AVP was added w bated without the anesthetic (controls). Cell supersion was passed through a flow cytomet<br>at 300-500 cells/sec. AVP was added without<br>interruption of the flow of cells. Fluorescence,<br>dual wavelengths, was used to calculate pension was passed<br>at 300–500 cells/see<br>interruption of the flo<br>dual wavelengths, w<br>ent [Ca<sup>2+</sup>],. Results finents are shown. dual wavelengths, was used to calculate apparent [Ca<sup>2+</sup>]. Results from typical individual experiments are shown.<br>**Fig. 2.** Effects of preincubation with halothane<br>on increases in [Ca<sup>2+</sup>], evoked by 10<sup>-7</sup> m AVP<br>in cultur

It [Ca<sup>2</sup>]. Hesults from typical incrviously experients are shown.<br>
Fig. 2. Effects of preincubation with halothane<br>
on increases in [Ca<sup>2+</sup>], evoked by 10<sup>-7</sup> M AVP<br>
in cultured A7r5 vascular smooth muscle cells. ients are shown.<br>**Fig. 2.** Effects of preincubation with halothane<br>on increases in [Ca<sup>2+</sup>], evoked by 10<sup>-7</sup> m AVP<br>in cultured A7r5 vascular smooth muscle cells.<br>The indo-1-loaded cells were incubated with **Fig. 2.** Effects of preincubation with halothane<br>on increases in  $[Ca^{2+}]$ , evoked by  $10^{-7}$  M AVP<br>in cultured A7r5 vascular smooth muscle cells.<br>The indo-1-loaded cells were incubated with<br>either 0.5, 1.25, or 2.0% halo Fig. 2. Effects of preincubation with halothane<br>on increases in  $[Ca^{2+}]$ , evoked by  $10^{-7}$  M AVP<br>in cultured A7r5 vascular smooth muscle cells.<br>The indo-1-loaded cells were incubated with<br>either 0.5, 1.25, or 2.0% halot Fig. 2. Effects of preincubation with halomane<br>on increases in  $[Ca^{2+}]}$ , evoked by  $10^{-7}$  M AVP<br>in cultured A775 vascular smooth muscle cells.<br>The indo-1-loaded cells were incubated with<br>either 0.5, 1.25, or 2.0% haloth on increases in [Car<sup>-</sup> ], evoked by 10 ' M AVP<br>in cultured A7r5 vascular smooth muscle cells.<br>The indo-1-loaded cells were incubated with<br>either 0.5, 1.25, or 2.0% halothane for 10 min<br>or incubated without the anesthetic in cultured Arrs vascular smooth muscle cells.<br>The indo-1-loaded cells were incubated with<br>or incubated without the anesthetic (controls).<br>Cell suspension was passed through a flow<br>cytometer at 300-500 cells/sec. AVP was<br>a The line-1-loaded casts were linculated with<br>either 0.5, 1.25, or 2.0% halothane for 10 min<br>or incubated without the anesthetic (controls).<br>Cell suspension was passed through a flow<br>cytometer at 300-500 cells/sec. AVP was<br> Fluorescence at the mass and the method of included without the anesthetic (controls).<br>Cell suspension was passed through a flow<br>cytometer at 300–500 cells/sec. AVP was<br>added without interruption of the flow of cells.<br>Fluo or includated without the anesthetic (controls).<br>Cell suspension was passed through a flow<br>cytometer at 300-500 cells/sec. AVP was<br>added without interruption of the flow of cells.<br>Fluorescence, at dual wavelengths, was us Less suspension was passed triough and ded without interruption of the flow of Fluorescence, at dual wavelengths, was to calculate apparent  $[Ca^{2+}]$ . Results fro ical individual experiments are shown. added without interruption of the flow of cells.<br>
The added without interruption of the flow of cells.<br>
Fluorescence, at dual wavelengths, was used<br>
to calculate apparent  $[Ca^{2+}]$ . Results from typ-<br>
ical individual exper

**E**<br>result from receptor-operated Ca<sup>2+</sup> influx, because it was not<br>inhibited by  $10^{-6}$  M diltiazem, a voltage-operated Ca<sup>2+</sup> channel<br>blocker. Sustained phase [Ca<sup>2+</sup>], was  $183 \pm 27$  nM in control<br>cells and  $174 \pm 24$  result from receptor-operated Ca<sup>2+</sup> influx, because it was not<br>inhibited by  $10^{-6}$  M diltiazem, a voltage-operated Ca<sup>2+</sup> channel<br>blocker. Sustained phase  $[Ca^{2+}]_i$  was  $183 \pm 27$  nM in control<br>cells and  $174 \pm 24$  nM result from receptor-operated Ca<sup>2+</sup> influx, because it was not<br>inhibited by  $10^{-6}$  M diltiazem, a voltage-operated Ca<sup>2+</sup> channel hibit<br>blocker. Sustained phase  $[Ca^{2+}]$ ; was  $183 \pm 27$  nM in control inhi<br>cells and  $174$ blocker. Sustained phase  $[Ca^{2+}]$ ; was  $183 \pm 27$  nM in control in cells and  $174 \pm 24$  nM in the presence of diltiazem. In addition, from pretreatment with 1 nM  $La^{3+}$ , an inorganic nonspecific  $Ca^{2+}$  plateau phase fro cells and 174  $\pm$  24 nM in the presence of diltiazem. In addition, from pretreatment with 1 nM  $La^{3+}$ , an inorganic nonspecific  $Ca^{2+}$  plackennel blocker, decreased the sustained phase from  $184 \pm 28$  exp nM to  $45 \pm 1$ increased  $[Ca^{2+}]_i$  by only 49  $\pm$  11 nM.

channel blocker, decreased the sustained phase from  $184 \pm 28$  experiments are presented in Fig. 5. Comparisons of integrated<br>nM to  $45 \pm 19$  nM, suggesting that the plateau phase was caused areas under the time-response  $\frac{1}{10}$  to calculate apparent  $[Ca^{2+}]$ . Results from typical individual experiments are shown.<br>Preincubation with 1.5% halothane resulted in marked in-<br>hibition of the Ca<sup>2+</sup> release transient but had only modest<br>inhib in the second superiments are shown.<br>
Freincubation with 1.5% halothane resulted in marked in-<br>
hibition of the Ca<sup>2+</sup> release transient but had only modest<br>
inhibitory effects on the second sustained response resulting<br> Freincubation with 1.5% halothane resulted in marked in-<br>hibition of the Ca<sup>2+</sup> release transient but had only modest<br>inhibitory effects on the second sustained response resulting<br>from Ca<sup>2+</sup> influx. The effects of haloth Preincubation with 1.5% halothane resulted in marked in hibition of the  $Ca^{2+}$  release transient but had only modes inhibitory effects on the second sustained response resultin from  $Ca^{2+}$  influx. The effects of halotha multion of the Ca<sup>2+</sup> release transient but had only modest<br>inhibitory effects on the second sustained response resulting<br>from Ca<sup>2+</sup> influx. The effects of halothane on the Ca<sup>2+</sup> influx<br>plateau are shown in Table 4. The inhibitory effects on the second sustained response resulting<br>from  $Ca^{2+}$  influx. The effects of halothane on the  $Ca^{2+}$  influx<br>plateau are shown in Table 4. The results of typical individual<br>experiments are presented i from Ca<sup>2+</sup> influx. The effects of halothane on the Ca<sup>2+</sup> influx<br>plateau are shown in Table 4. The results of typical individual<br>experiments are presented in Fig. 5. Comparisons of integrated<br>areas under the time-respons plateau are shown in Table 4. The results of typical individual<br>experiments are presented in Fig. 5. Comparisons of integrated<br>areas under the time-response curves indicated that halothane<br>inhibited the response due to  $Ca$ experiments are presente<br>areas under the time-respinhibited the response d<br>0.0001), whereas the subs<br>by only  $15 \pm 4\%$  ( $p < 0.0$ 



Fig. 3. Percentage of inhibition by halothane of increases in  $[Ca^{2+}]$ induced by 10<sup>-9</sup> and 10<sup>-7</sup> M AVP in cultured A7r5 vascular smooth muscle cells. Left, cells were incubated in DMEM, with or without 0.5, 1.25, or 2.0% halothane, for 10 min and then stimulated with AVP. Right, cells were incubated with or without halothane in Ca<sup>2+</sup>-free MEM plus 0.5 mm EGTA, to decrease extracellular [Ca<sup>2+</sup>]. (On stimulation with AVP, the increase in  $[Ca^{2+}]$ , was due to  $Ca^{2+}$  release from intracellular stores.) Integrated areas under time-response curves were used to indicate changes in  $[Ca^{2+}]_i$ .  $n = 8-13$  pairs for each experiment. \*,  $p <$  $0.01;$  t,  $p < 0.0001$ .



Fig. 4. Effects of preincubation with halothane on increases in  $[Ca^{2+}]$ evoked by AVP (left, 10<sup>-9</sup> M; right, 10<sup>-7</sup> M in A7r5 cultured vascular smooth muscle cells. The indo-1-loaded cells were incubated either with or without 1.5% halothane in Ca<sup>2+</sup>-free MEM plus 0.5 mm EGTA, to decrease extracellular [Ca<sup>2+</sup>]. Apparent [Ca<sup>2+</sup>], was measured using dualwavelength sampling from cells imaged during flow cytometry. The increase in [Ca<sup>2+</sup>], represents Ca<sup>2+</sup> release from intracellular stores evoked by AVP. Tracings from typical experiments are presented.

Effects of preincubation with halothane on increases in  $[Ca<sup>2+</sup>]$  evoked by thapsigargin. The effects of preincubation with halothane on the Ca<sup>2+</sup> content of intracellular stores was assessed by suspending the cells in  $Ca<sup>2+</sup>$ -free medium plus EGTA and stimulating them with thapsigargin. [This agent discharges  $Ca^{2+}$  from  $Ins(1,4,5)P_3$ -sensitive stores, with the magnitude of the  $Ca^{2+}$  response reflecting the amount of releasable  $Ca^{2+}$  present (17).] Thapsigargin induced a gradual increase in  $[Ca^{2+}]$ , with maximum levels occurring at approximately 200 sec, followed by a gradual return towards base line. The amplitude of the increase in  $[Ca^{2+}]_i$  was 59  $\pm$  3 nM in control cells and decreased to  $41 \pm 2$  nM in cells preincubated with 1.5% halothane ( $p < 0.0001$ ). Comparisons of integrated areas under time-response curves indicated that halothane inhibited the response by 31  $\pm$  5% ( $p$  < 0.001). Tracings from typical individual experiments are shown in Fig. 6.

Effects of preincubation with halothane on increases in [Ca<sup>2+</sup>], evoked by PDGF. Stimulation of untreated cells with 3.3  $\times$  10<sup>-9</sup> M PDGF resulted in increases in [Ca<sup>2+</sup>]; with maximum levels occurring at approximately 100 sec, followed by return towards base line. The responses were inhibited in cells preincubated with 1.5% halothane. Base-line, peak, and 250-sec  $[Ca^{2+}]$  levels are shown in Table 5. Comparison of integrated areas under the time-response curves indicated halothane decreased the PDGF-induced  $[Ca^{2+}]$ ; responses by 43  $\pm$  5% ( $p < 0.0001$ ). Tracings from typical individual experiments are presented in Fig. 7.

Effects of preincubation with halothane on increases in total inositol phosphates evoked by AVP. The aim was to determine whether preincubation with halothane inhibited increases in inositol phosphates evoked by  $10^{-7}$  M AVP. Total inositol phosphate levels were first measured 2 min before addition of AVP when results demonstrated that resting inositol phosphate levels in quiescent cells did not differ between cells treated with halothane and untreated cells. AVP was then added and induced a sustained increase in total inositol phosphate levels. (Degradation of inositol phosphates was inhibited due to the presence of LiCl.) The response was attenuated after pretreatment of cells with both 1 and 2% halothane. Levels of inositol phosphates in cells untreated with either AVP or halothane remained stable during the experiment and provided time control data. Comparisons of integrated areas under the time-response curves, calculated using time-control measurements as base-line, indicated that 1% halothane inhibited the AVP-induced increase in inositol phosphates by  $46 \pm 15\%$  (p  $<$  0.02) and that 2% halothane inhibited the response by 61  $\pm$ 19% ( $p < 0.02$ ). The results are displayed in Fig. 8.

# **Discussion**

The mechanisms of action of anesthetics are not understood. Current theories predict that the anesthetic state and related side effects such as vasodilatation may arise from interference with cell signaling (5). Results from the current experiments suggest that agonist-induced increases in  $[Ca^{2+}]_i$  and in inositol phosphates, two uniquely important steps in cell signaling, are inhibited by halothane. Preincubation with clinically relevant concentrations of halothane resulted in attenuation of the

# **TABLE 4**

Effects of preincubation with halothane upon Ca<sup>2+</sup> release from intracellular stores and upon Ca<sup>2+</sup> influx, both evoked by 10<sup>-7</sup> M AVP The indo-1-loaded cells were preincubated for 10 min, with or without halothane, in Ca<sup>2+</sup>-free culture medium plus 0.5 mm EGTA. Apparent [Ca<sup>2+</sup>], was obtained using flow cytometry. AVP was injected into the cell suspension without interruption of the flow of cells. After completion of the initial transient, which represents Ca<sup>2+</sup> release from intracellular stores, 5 mm Ca<sup>2+</sup> was added to the cell suspension in the continued presence of AVP. The second response represents Ca<sup>2+</sup> influx.

Halothane		Peak $[Ca2+]$ due to $Ca2+$ release		Peak $[Ca^{2+}]$ due to $Ca^{2+}$ influx		$[Ca2+]$ at 250 sec during $Ca2+$ influx	
concentration	п	Control	Halothane	Control	Halothane	Control	Halothane
%		nM		nM		nм	
1.5	12	$449 \pm 28$	$184 \pm 18^{\circ}$	$188 \pm 24$	$150 \pm 17^{\circ}$	$152 \pm 9$	$132 \pm 15^b$

 $p < 0.0001$ , in comparison with control.

 $\dot{p}$  < 0.01, in comparison with control.



Fig. 5. Effects of preincubation with 1.5% halothane on increases in  $[Ca<sup>2+</sup>]$  due to  $Ca<sup>2+</sup>$  release and  $Ca<sup>2+</sup>$  influx in A7r5 cultured vascular smooth muscle cells stimulated with  $10^{-7}$  M AVP. The indo-1-loaded cells were incubated either with or without halothane in Ca<sup>2+</sup>-free MEM plus 0.5 mm EGTA, to decrease extracellular [Ca<sup>2+</sup>]. Apparent [Ca<sup>2+</sup>], was measured using dual-wavelength sampling of light emitted from cells during flow cytometry. The initial peak (left) represented [Ca<sup>2+</sup>], increase due to Ca<sup>2+</sup> release from intracellular stores. The second sustained increase occurred when 5 mm  $Ca<sup>2+</sup>$  was added after completion of the initial transient, in the continued presence of AVP, and represented Ca<sup>2+</sup> influx. Tracings from typical individual experiments are shown.



Fig. 6. Effects of preincubation with 1.5% halothane on increases in  $^{\text{+}}$ ], elicited in cultured A7r5 vascular smooth muscle cells by 5  $\times$  10<sup>-5</sup> M thapsigargin. [Thapsigargin is believed to elevate  $[Ca<sup>2+</sup>]$  by discharging  $Ca^{2+}$  from  $Ins(1,4,5)P_3$ -sensitive intracellular  $Ca^{2+}$  stores.] Cells were suspended in Ca<sup>2+</sup>-free MEM plus 0.5 mm EGTA to decrease extracellular [Ca<sup>2+</sup>]. Results from an individual experiments are shown.

 $[Ca^{2+}]$  responses evoked in cultured vascular smooth muscle cells by the vasoactive hormone AVP. The anesthetic effect was dose dependent and the dose-response relationship was surprisingly steep. The effects of halothane on  $Ca^{2+}$  release were apparently much greater than those on  $Ca^{2+}$  influx. The actions of the anesthetic on  $Ca^{2+}$  homeostasis cannot be explained solely by depletion of  $Ca^{2+}$  from intracellular stores, because preincubation with halothane resulted in only a moderate inhibition of the  $[Ca^{2+}]$ , responses elicited by thapsigargin in cells suspended in Ca<sup>2+</sup>-free medium. Halothane inhibited inositol phosphate formation elicited by AVP, suggesting an additional action by which the anesthetic may alter agonistinduced  $[Ca^{2+}]$  responses. Halothane actions were not specific solely to responses elicited by AVP, which acts via a G proteinlinked signaling system, because the anesthetic also inhibited those evoked by PDGF, an agonist that elevates  $[Ca^{2+}]$ , via receptor-latent tyrosine kinase activity.

Vasoactive hormones, including AVP, elevate inositol phosphates and  $[Ca^{2+}]$ ; by activating a signaling system consisting of cell surface receptors that are presumed to possess a topography of seven membrane-spanning domains and are coupled to effector enzymes and ion channels via intermediary G proteins (12). Phosphoinositide-specific phospholipase C is the membrane-bound enzyme responsible for hydrolysis of the major substrate, phosphatidylinositol 4,5-bisphosphate, to form the cytosolic second messengers  $Ins(1,4,5)P_3$  and diacylgiveerol (13). The  $Ins(1,4,5)P_3$  receptor on membranes enclosing intracellular Ca<sup>2+</sup> stores has been purified and cloned, and its activation results in discharge of  $Ca^{2+}$  from portions of the endoplasmic reticulum. In some cells,  $Ins(1,4,5)P_3$  is believed to act in conjunction with its metabolite inositol 1.3.4.5-tetrakisphosphate in regulating  $Ca^{2+}$  movement (13). In contrast to release of  $Ca^{2+}$ , an understanding of the mechanisms regulating  $Ca<sup>2+</sup>$  influx has remained more elusive (15, 17).

Halothane had a marked inhibitory effect on increases in  $[Ca<sup>2+</sup>]$  caused by  $Ca<sup>2+</sup>$  release. This action could be explained by interference with the signaling system responsible for  $Ca^{2+}$ release or, alternatively, by depletion of Ca<sup>2+</sup> from intracellular stores. The current results suggest that both occur. In the current study, the effects of halothane upon Ca<sup>2+</sup> content of intracellular storage pools were addressed by preincubating cells with the anesthetic and then stimulating them with thapsigargin. The increase in  $[Ca^{2+}]$  elicited by this agent results from  $Ca^{2+}$  discharge from  $Ins(1,4,5)P_3$ -sensitive storage sites (17), with the magnitude of the response reflecting releasable  $Ca<sup>2+</sup>$  remaining in the pools. Preincubation with halothane inhibited the response, suggesting that Ca<sup>2+</sup> storage is impaired by the anesthetic. However, the magnitude of the depletion of  $Ca<sup>2+</sup>$  was not sufficiently great to completely account for inhibition of the  $[Ca^{2+}]$  responses evoked by AVP and by PDGF. The results are in agreement with observations made in cardiac tissue, where halothane is thought to deplete  $Ca^{2+}$  from the sarcoplasmic reticulum by decreasing uptake via the Ca<sup>2+</sup>-ATPase pump (16, 24) or by discharging Ca<sup>2+</sup> via caffeinesensitive Ca<sup>2+</sup>-release channels (25). Less is known concerning the actions of halothane in vascular smooth muscle, although halothane promotes  $Ca^{2+}$  loss from caffeine-sensitive stores in rabbit aortic strips (26). The current results indicate that Ins $(1,4,5)P_3$ -sensitive Ca<sup>2+</sup> stores in vascular smooth muscle cells are susceptible to depletion by halothane.

The absence of immediate and pronounced increases in  $[Ca<sup>2+</sup>]$ ; upon acute addition of halothane to quiescent cells suggests that the anesthetic does not discharge  $Ca^{2+}$  from stores in a rapid manner. This absence of immediate effect was surprising, because it contrasts sharply with responses reported

TABLE 5

Effects of preincubation with halothane upon base-line [Ca<sup>2+</sup>], and upon increases in [Ca<sup>2+</sup>], evoked by 3.3 x 10<sup>-9</sup> M PDGF

Base-line [Ca <sup>2+</sup> ]		Peak [Ca <sup>2+</sup> ]		$[Ca2+]$ at 250 sec	
Control	Halothane	Control	Halothane	Control	Halothane
nм		nu		nM	
$50 \pm 1$	$46 \pm 1^{\circ}$	$390 \pm 17$	$217 \pm 19^{\circ}$	$102 \pm 5$	$78 \pm 4^b$

 $p < 0.01$ , in comparison with control.

 $\degree$   $p$  < 0.0001, in comparison wtih control.



Fig. 7. Effects of preincubation with 1.5% halothane on increases in ever,<br>[Ca<sup>2+</sup>] in cultured A7r5 vascular smooth muscle cells stimulated with 3.3<br>x 10<sup>-9</sup> M PDGF. The indo-1-loaded cells were incubated either with or<br>w **Example 10** methods and **Fig. 7.** Effects of preincubation with 1.5% halothane on increases in  $[Ca^{2+}j]$  in cultured A7r5 vascular smooth muscle cells stimulated with 3.3  $\div$  10<sup>-9</sup> m PDGF. The indo-1-loaded cells were Fig. 7. Effects<br> $[Ca^{2+}$ <sub>1</sub> in culture<br> $\times$  10<sup>-9</sup> M PDGF<br>without halothar<br>are presented.



Fig. 8. Effects of halothane on inositol phosphate formation stimulated<br>Fig. 8. Effects of halothane on inositol phosphate formation stimulated tion.<br>which 10<sup>-7</sup> M AVP in cultured A7r5 vascular smooth muscle cells. The H Fig. 8. Errects or natomane on inostrol phosphate formation stimulated with  $10^{-7}$  M AVP in cultured A7r5 vascular smooth muscle cells. The myo-[<sup>3</sup>H]inositol-labeled cells were incubated either with or without 1% or  $2\$ with 10  $^{\circ}$  M AVP in cultured A/15 vascular smooth muscle cells. The myo-[<sup>3</sup>H]inositol-labeled cells were incubated either with or without 1% contexts and the main timulated with AVP. (Control cells were exposed to ne myo-["H]inosito-labeled cells were included either with or without 1%<br>or 2% halothane, for 10 min, and then stimulated with AVP. (Control<br>cells were expoosed to neither halothane nor AVP.) Comparison of<br>responses evoked b or 2% natomane, for 10 min, and then stime<br>cells were exposed to neither halothane no<br>responses evoked by AVP alone versus respo<br>preincubation with halothane indicated statistic<br>effects at each anesthetic concentration (co responses evoked by AVP alone versus responses evoked by AVP after<br>preincubation with halothane indicated statistically significant depressant<br>effects at each anesthetic concentration (comparison of slopes,  $\rho < 0.05$ .<br>Co

effects at each anesthetic concentration (comparison of slopes,  $\rho < 0.05$ .<br>Comparison of integrated areas,  $\rho < 0.02$ ).<br>in many types of cells, including those originating from heart<br>(10), liver (27), skeletal muscle (28 Comparison or integrated areas,  $p < 0.02$ ).<br>
in many types of cells, including those originating from heart<br>
(10), liver (27), skeletal muscle (28), blood (22), and brain (29),<br>
where halothane acutely elevates  $[Ca^{2+}]_i$ in many types of cells, including those originating from heart<br>
(10), liver (27), skeletal muscle (28), blood (22), and brain (29),<br>
where halothane acutely elevates  $[Ca^{2+}]_i$ . Increase in  $[Ca^{2+}]_i$  is<br>
a marker of cell (10), liver (27), skeletal muscle (28), blood (22), and brain (29),<br>where halothane acutely elevates  $[Ca^{2+}]_i$ . Increase in  $[Ca^{2+}]_i$  is<br>a marker of cell activation. In neuronal cells, increased  $[Ca^{2+}]_i$ <br>has been asso where halothane acutely elevates  $[Ca^+]_i$ . Increase in  $[Ca^2']_i$  is<br>a marker of cell activation. In neuronal cells, increased  $[Ca^2^+]_i$ <br>has been associated with activation of  $K^+$  channels, and the<br>resulting depression a marker of central activation. In heuronal cents, increased [Ca ] is<br>has been associated with activation of  $K^+$  channels, and the<br>resulting depression of excitability has served as a model of<br>general anesthesia (6, 29) has been associated with activation of K<sup>-</sup> channels, and the resulting depression of excitability has served as a model of general anesthesia (6, 29). However, gradual depletion of Ca<sup>2+</sup> from internal stores noted in the general anesthesia (o, 29). However, gradual defrom internal stores noted in the current experient than abrupt acute discharge of  $Ca^{2+}$  into the cyto in other cell types, is more compatible with the effects of halothane an abrupt acute discharge of  $Ca^{2+}$  into the cytoplasm observed<br>other cell types, is more compatible with the *in vivo* vascular<br>fects of halothane, which involve vasodilatation.<br>Attenuation of AVP-induced  $Ca^{2+}$  releas

Final abrupt acute unscharge of Ca and the cytopiasm observed<br>in other cell types, is more compatible with the *in vivo* vascular<br>effects of halothane, which involve vasodilatation.<br>Attenuation of AVP-induced  $Ca^{2+}$  rele Attenuation of AVP-induced  $Ca^{2+}$  release may be also explained by the inhibitory effect of halothane on AVP-induced inositol phosphate formation, a finding that represents a previously unreported action of volatile anes Muslim 45 section of AVP -induced Callierate may be also ex-<br>plained by the inhibitory effect of halothane on AVP-induced<br>inositol phosphate formation, a finding that represents a pre-<br>was present 45 sec after stimulation individually unreported action of volatile anesthetics. The effect<br>was present 45 sec after stimulation with AVP and persisted at<br>8 min when inositol phosphate formation was maximal (due to<br>inhibited breakdown in the prese whously unreported action of volatile anesthetics. The enections of the sum as present 45 sec after stimulation with AVP and persisted a 8 min when inositol phosphate formation was maximal (due the inhibited breakdown in t 8 min when inositol phosphate formation was maximal (due to inhibited breakdown in the presence of LiCl). Although the experiments did not define the locus of the actions of halothane, the results may be explained by direc pase C, by depletion of phosphatidylinositol 4,5-bisphosphate as <sup>a</sup> substrate for phospholipase C, or by increased breakdown experiments and not define the focus of the actions of halothane,<br>the results may be explained by direct inhibition of phospholi-<br>pase C, by depletion of phosphatidylinositol 4,5-bisphosphate<br>as a substrate for phospholipa the results hay be explained by direct inhibition of phosphon-<br>pase C, by depletion of phosphatidylinositol 4,5-bisphosphate<br>as a substrate for phospholipase C, or by increased breakdown<br>of inositol phosphates. Enzymes are

**Fig. 7.** Effects of preincubation with 1.5% halothane on increases in [Ca<sup>2+</sup>] in cultured A7r5 vascular smooth muscle cells stimulated with 3.3 mosticl phospholipids, the levels of total inositol phosphates of preincuba exist, with the most well characterized example being inhibition<br>of the light-emitting enzyme firefly luciferase by halothane (5). exist, with the most well characterized example being inhibition<br>of the light-emitting enzyme firefly luciferase by halothane (5).<br>In this instance, the anesthetic competes with the subtrate exist, with the most well characterized example being inhibition<br>of the light-emitting enzyme firefly luciferase by halothane (5).<br>In this instance, the anesthetic competes with the subtrate<br>firefly luciferin for binding s exist, with the most well characterized example being inhibition<br>of the light-emitting enzyme firefly luciferase by halothane (5).<br>In this instance, the anesthetic competes with the subtrate<br>firefly luciferin for binding s **stast,** with the most wen characterized example being inhibition of the light-emitting enzyme firefly luciferase by halothane (5). In this instance, the anesthetic competes with the subtrate firefly luciferin for binding in this instance, the anesthetic competes with the subtrate<br>firefly luciferin for binding sites on luciferase. In the current<br>study, the proposed relationship between inhibition of inositol<br>phosphate formation and attenua bloogues in an interest in minimized of messic<br>phosphate formation and attenuation of  $Ca^{2+}$  release should b<br>viewed with a degree of caution, because total inositol pho<br>phates were measured rather than concentrations<br>In phosphate formation and attenuation of Ca<sup>1</sup> release should be<br>viewed with a degree of caution, because total inositol phos-<br>phates were measured rather than concentrations of<br>Ins(1,4,5)P<sub>3</sub>, the messenger responsible for viewed with a degree of caution, because total inositol photos were measured rather than concentrations (Ins(1,4,5)P<sub>3</sub>, the messenger responsible for Ca<sup>2+</sup> release. However, because phosphatidylinositol 4,5-biphosphate d phaces were measured rather than concentrations of<br>Ins(1,4,5)P<sub>3</sub>, the messenger responsible for  $Ca^{2+}$  release. How-<br>ever, because phosphatidylinositol 4,5-biphosphate degradation<br>results in the formation of inositol ph ins(1,4,0)F<sub>3</sub>, the messenger responsible for Ca Telease. However, because phosphatidylinositol 4,5-biphosphate degradation results in the formation of inositol phosphates via Ins(1,4,5)P<sub>3</sub>, with only small amounts being results in the formation of inositol phosphates via Ins(1,4,5)P<sub>3</sub>, with only small amounts being formed by hydrolysis of other inositol phospholipids, the levels of total inositol phosphates should reflect concentrations sults in the formation of mositor phosphates via  $ins(1,4,5)F_3$ ,<br>th only small amounts being formed by hydrolysis of other<br>ositol phospholipids, the levels of total inositol phosphates<br>ould reflect concentrations of  $Ins(1,4$ 

with only small amounts being formed by hydrolysis of other<br>inositol phospholipids, the levels of total inositol phosphates<br>should reflect concentrations of  $\text{Ins}(1,4,5)P_3$  (30).<br>In contrast to the marked inhibitory effe mositol phospholipids, the levels of total inositol phosphates<br>should reflect concentrations of  $\text{Ins}(1,4,5)P_3$  (30).<br>In contrast to the marked inhibitory effect of halothane on<br>AVP-induced Ca<sup>2+</sup> release, Ca<sup>2+</sup> influx In contrast to the marked inhibitory effect of halothane on<br>AVP-induced Ca<sup>2+</sup> release, Ca<sup>2+</sup> influx was apparently only<br>modestly decreased by the anesthetic. This observation was<br>surprising because, in many types of cel  $AVP$ -induced Ca<sup>2+</sup> release, Ca<sup>2+</sup> influx was apparently only modestly decreased by the anesthetic. This observation was surprising because, in many types of cells,  $Ca^{2+}$  release and  $Ca^{2+}$  influx are tightly coupled, modestry decreased by the anesthetic. This observation was<br>surprising because, in many types of cells,  $Ca^{2+}$  release and<br> $Ca^{2+}$  influx are tightly coupled, with depletion of  $Ca^{2+}$  from<br>intracellular stores serving to surprising because, in many types of cells, Ca<sup>2+</sup> release and  $Ca^{2+}$  influx are tightly coupled, with depletion of  $Ca^{2+}$  from intracellular stores serving to initiate  $Ca^{2+}$  entry (17). An alternative but related mod intracellular stores serving to initiate  $Ca^{2+}$  entry (17). An alternative but related model of influx control suggests that activated receptors directly open cell membrane  $Ca^{2+}$  channels (15). The current divergent ef atternative but related model of minds control suggests that<br>activated receptors directly open cell membrane  $Ca^{2+}$  channels<br>(15). The current divergent effects of halothane on release<br>versus influx may suggest that dire The carrent divergent enects of nanothane on release<br>versus influx may suggest that direct opening of channels b<br>receptors, followed by  $Ca^{2+}$  influx, is not sensitive to inhibition<br>by the anesthetic. The current results preceptors, followed by  $Ca^{2+}$  influx, is not sensitive to inhibition<br>by the anesthetic. The current results differ from observations<br>made in isolated ventricular myocytes, where halothane de-<br>pressed the inward  $Ca^{2+}$  by the anesthetic. The current results differ from observation<br>by the anesthetic. The current results differ from observation<br>made in isolated ventricular myocytes, where halothane<br>pressed the inward  $Ca^{2+}$  current (9); tion. Halothane inhibited increases in [Ca<sup>2+</sup>], elicited by PDGF,<br>Halothane inhibited increases in [Ca<sup>2+</sup>], elicited by PDGF,<br>an agonist that acts via an intracellular effector system that<br>differs from the signaling pathway ac

were evoked by depolarization rather than by receptor activa-<br>tion.<br>Halothane inhibited increases in  $[Ca^{2+}]_i$  elicited by PDGF<br>an agonist that acts via an intracellular effector system that<br>differs from the signaling pat Halothane inhibited increases in  $[Ca^{2+}]$ ; elicited by PDG an agonist that acts via an intracellular effector system the differs from the signaling pathway activated by pressor he mones. Receptors for this growth factor p Halothane inhibited increases in [Ca<sup>21</sup>], elicited by PDGF,<br>an agonist that acts via an intracellular effector system that<br>differs from the signaling pathway activated by pressor hor-<br>mones. Receptors for this growth fact mones. Receptors for this growth factor possess a single trans-<br>membrane region and a cytoplasmic domain that, on receptor<br>dimerization, exhibits tyrosine kinase activity, resulting in<br>phosphorylation of phospholipase C, p actions of halothane are not restricted solely to responses membrane region and a cytoplasmic domain that, on receptor<br>dimerization, exhibits tyrosine kinase activity, resulting in<br>phosphorylation of phospholipase C, possibly without the in-<br>volvement of G proteins (18). The result dimerization, exhibits tyrosine kinase activity, resulting in<br>phosphorylation of phospholipase C, possibly without the in-<br>volvement of G proteins (18). The results indicate that the<br>actions of halothane are not restricted phosphorylation of phosphorylation<br>volvement of G proteins (1<br>actions of halothane are n<br>evoked by AVP but include<br>different signaling pathways<br>In conclusion, it is tempt In conclusion, it is tempting to speculate that the<br>ideas of halothane are not restricted solely to responses<br>oked by AVP but include responses elicited via distinctly<br>fferent signaling pathways.<br>In conclusion, it is tempt

actions of halothane are not restricted solety to responses<br>evoked by AVP but include responses elicited via distinctly<br>different signaling pathways.<br>In conclusion, it is tempting to speculate that, in humans<br>undergoing a different signaling pathways.<br>In conclusion, it is tempting to speculate that, in humans<br>undergoing anesthesia, the vasodilator effects of halothane<br>result from a direct effect of the anesthetic on  $Ca^{2+}$  homeostasis<br>in different signaling pathways.<br>In conclusion, it is tempting to speculate that, in humans<br>undergoing anesthesia, the vasodilator effects of halothane<br>result from a direct effect of the anesthetic on  $Ca^{2+}$  homeostasis<br>in In conclusion, it is tempting to speculate that, in numalis<br>undergoing anesthesia, the vasodilator effects of halothane<br>result from a direct effect of the anesthetic on  $Ca^{2+}$  homeostasis<br>in vascular smooth muscle cells. undergoing anesthesia, the vasodilator effects of halotharesult from a direct effect of the anesthetic on Ca<sup>2+</sup> homeostain vascular smooth muscle cells. However, the cell culture moshould be viewed with a degree of cautio

### **Acknowledgments**

with which cell culture experiments represent in vivo physio-<br>logical events always remains uncertain.<br>Acknowledgments<br>The authors would like to thank Garth Powis, James Lechleiter, and Carol<br>Williams, from the Departments Acknowledgments<br>The authors would like to thank Garth Powis, James Lechleiter, and Carol<br>Williams, from the Departments of Pharmacology and Immunology, for their<br>advice concerning study design and for reviewing the manuscr Acknowledgments<br>The authors would like to thank Garth Powis, James Lechleiter, and Caro<br>Williams, from the Departments of Pharmacology and Immunology, for the<br>advice concerning study design and for reviewing the manuscript The authors would like to thank Garth Powin<br>Williams, from the Departments of Pharmacolo<br>advice concerning study design and for reviewing<br>assistance of Janet Beckman is greatly appreciate

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