AN ANALYSIS OF THE DEPRESSOR AND PRESSOR EFFECTS OF TRYPTAMINE IN THE CHICKEN

JOHN NELSON EBLE

Biomedical Research Department, Pitman-Moore Company, Indianapolis, Indiana, A Division of The Dow Chemical Company

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The potentiation of the pressor effect of tryptamine in the dog by monoamine oxidase inhibitors has been shown by Goldberg (1959) and Burford and Walaszek (1960). That tryptamine is a depressor in the chicken has been reported by Powell *et al.* (1955) and more recently by Buñag and Walaszek (1961). In experiments to determine if MAO inhibitors would, in the chicken, potentiate the depressor response to tryptamine as they potentiate the pressor response in the dog, we were surprised to find that instead, they caused a reversal of the depressor effect. This finding prompted the following study of both the depressor and pressor (post-MAO inhibitor) actions of tryptamine in the chicken.

The data presented suggest that the hypotensive effect is the result of vasoconstriction. Thus, a vasoconstriction of the pulmonary bed and the resultant decreased blood return have a greater effect on systemic blood pressure than the increase in resistance resulting from the simultaneous peripheral vasoconstriction.

METHODS. More than 100 chickens of either sex, and of various breeds, were used in these experiments; weights ranged from 1.4 to 3.9 kg. Birds were anesthetized with intravenous injections of pentobarbital sodium, either alone or 10 minutes after intramuscular administration of a mixture of chloral hydrate and magnesium sulfate as suggested by Jordan *et al.* (1960). With the latter method, the dose of pentobarbital sodium was 15 to 20 mg/kg and 3 of the 39 premedicated chickens required no further anesthetic. We usually use pentobarbital alone (30 to 40 mg/kg) with supplemental doses of 4 to 6 mg/kg as needed. The chickens' eyes were shielded from the light with a paper towel or a small fold of aluminum foil. The chickens were usually very lightly anesthetized, and occasionally raised their heads from the table with their eyes open. None of the above variables were observed to have consistent or important effects on results.

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Salts of the following drugs were used: tryptamine hydrochloride, phenoxybenzamine, histamine diphosphate, atropine sulfate, atropine methylnitrate, *l*-epinephrine bitartrate, *l*-norepinephrine *d*-bitartrate, *dl*-ephedrine hydrochloride, dihydroergotamine methanesulfonate, tolazoline hydrochloride, cyproheptadine hydrochloride monohydrate, acetylcholine chloride, cocaine hydrochloride, tranylcypromine, narcotine hydrochloride, polyvinylpyrrolidone (PVP) and 48/80.

Blood pressure was recorded from a cannulated ischiadic artery by means of a Statham transducer. In some experiments, the heart rate was recorded using a Grass cardiotachometer, but usually, the chicken heart rate exceeded the range of this instrument which is 300 beats/minute. Drug injections were made *via* a polyethylene tube passed 3 to 4 cm into the ischiadic vein.

Perfusion studies. The left leg in 11 chickens was perfused with blood from the abdominal aorta using a constant flow Sigmamotor pump and the perfusion pressure was measured with a Statham transducer. Flow was adjusted so as to approximate systemic pressure and varied from 12 to 20 ml/minute. The volume of the tubing between the abdominal aorta and the femoral artery was approximately 15 ml so that there was a 30- to 60-second delay between the systemic effect and the peripheral effect of an intravenously administered drug. This delay made it possible to distinguish between direct and neurogenic effects. Heparin was used as an anticoagulant.

Right ventricular pressure measurements. The right ventricle was catheterized *via* the right jugular vein. Placement was carefully verified by autopsy but was initially determined by the measurement of diastolic (0–3 mm Hg) and systolic (20–30 mm Hg) pressures. A Statham transducer was used to measure the pressure.

RESULTS. I. DEPRESSOR ACTION. A. *Cholinergic blockade.* In 7 chickens, 1 mg/kg of atropine totally blocked the depressor effects of 1 to 2 μg/kg of acetylcholine. This dose of atropine had no effect on the depressor responses of the chicken to tryptamine or histamine, but markedly

potentiated the pressor effects of *l*-epinephrine, as shown in figure 1. In 3 additional chickens, the dose of atropine was increased to 10 to 20 mg/kg and still no influence was observed on the tryptamine depressor response. Methylatropine was more potent; 0.1 mg/kg produced complete blockade of 2 to 8 μg/kg of acetylcholine (4 chickens). Five chickens were given 1 mg/kg of methylatropine with no effect on the depressor response to tryptamine.

A biphasic blood pressure response to tryptamine, initially pressor but predominantly depressor, occurred in approximately 1 of every 10 chickens in our series; only 3 of the untreated chickens had a predominantly pressor response.

B. *Adrenergic blockade.* Tolazoline is an effective adrenergic blocking agent in the chicken as first shown by Thompson and Coon (1948). In 5 chickens, a dose of 10 mg/kg of tolazoline blocked the pressor responses to *l*-epinephrine and greatly reduced the responses to norepinephrine. An additional dose of 10 mg/kg of tolazoline resulted in a blockade of the norepinephrine pressor responses in 4 of 5 chickens. These chickens showed either no change or an increased depressor response to both tryptamine and histamine, presumably because of the loss of buffer control. A transient fall in blood pressure followed by a prolonged moderate elevation was a characteristic response of the chickens to tolazoline in all 5 instances.

C. *Antihistaminic action.* In this study, phenoxybenzamine was found to be an antihistaminic as well as an antiadrenergic substance in the chicken.

The depressor effects of 10 to 30 μg/kg of histamine (4 chickens) were effectively antagonized by 10 to 15 mg/kg of phenoxybenzamine. The antihistaminic effect had a longer duration than the antiadrenergic effect.

Phenoxybenzamine was also found to be more consistent than the antihistaminic, diphenhydramine, in its blocking action of the tryptamine depressor effects since, in 5 chickens given phenoxybenzamine (5 to 15 mg/kg), the depressor effect of tryptamine was blocked and a pressor effect was unmasked. (Blockade of the depressor effect of histamine by phenoxybenzamine does not result in the unmasking of a pressor effect.) By contrast, in 9 chickens given 10 to 20 mg/kg of diphenhydramine, the depressor effect of tryptamine was unaffected in 2, only fleetingly blocked in 2 others, and in the remaining 5, the unmasking of the pressor action of tryptamine was not as clear-cut as with the phenoxybenzamine.

D. *Serotonin antagonism.* Cyproheptadine in doses of 200 to 500 μg/kg produced a 10- to 30-minute blockade of the depressor response to tryptamine in 4 chickens. Cyproheptadine also blocked the depressor action of histamine but, unlike the other antihistaminics, did not unmask a pressor response to tryptamine.

Dihydroergotamine in doses of 1 to 4 mg per

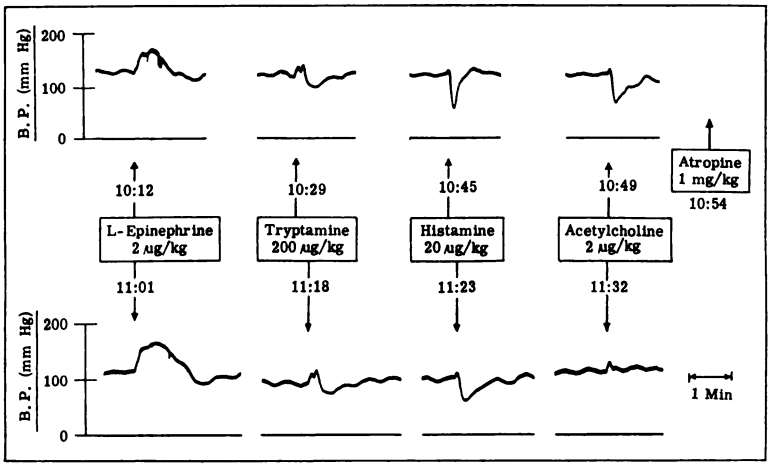

FIG. 1. The effect of atropine on the arterial blood pressure responses to intravenously administered epinephrine, tryptamine, histamine and acetylcholine.
Weight of chicken, 3.0 kg; intravenous pentobarbital anesthesia.

chicken blocked the depressor and unmasked the pressor response to tryptamine without affecting either histamine or acetylcholine. This was repeated in 5 chickens.

E. *Absence of tachyphylaxis.* Figure 2 shows responses to 7 consecutive doses of tryptamine (100 μg/kg) within 30 minutes without diminution of magnitude or duration. Such rapid repetitions of drug administration were tried in 8 additional chickens with the same result; as many as 15 consecutive doses have been given. These results may be compared with those of Natoff and Lockett (1957) who found the depressor response of the chicken to serotonin sufficiently stable to be used as a bioassay.

Cross-tachyphylaxis with 48/80 was not observed. Although tachyphylaxis to 48/80 develops quickly in the chicken (Buñag and Walaszek, 1962), it does not influence the depressor response to tryptamine as is shown in figure 3, and seen in 16 additional chickens with doses of 0.1 to 1.0 mg/kg of 48/80. In 2 of these experiments, the depressor response to tryptamine was blocked by the 48/80 for a 5- to 10-minute period immediately following the administration of 48/80. The intravenous infusion of 20 μg/kg per minute of histamine only transiently lowered base blood pressure and blocked the depressor action of tryptamine; this returned as soon as the infusion was stopped.

Narcotine, a histamine liberator in the dog but not in the cat (Weaver *et al.*, 1958), was given in doses of 4 mg/kg to 2 chickens and did not affect the blood pressure or the responses to tryptamine. PVP given in doses of 0.4 ml/kg of a 25% solution was also without effect in 3 chickens.

F. *Miscellaneous.* Dichloroisoproterenol had no influence on the depressor action of tryptamine

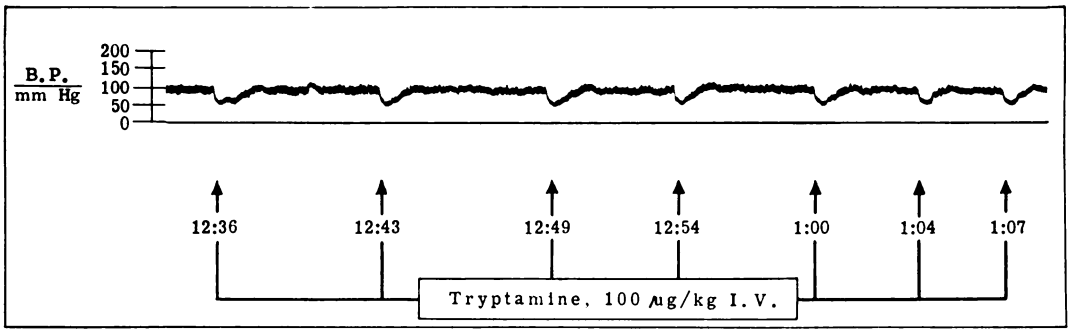

FIG. 2. The effect of repeated intravenous administration of tryptamine on arterial blood pressure. Male chicken, 2.7 kg; intravenous pentobarbital anesthesia.

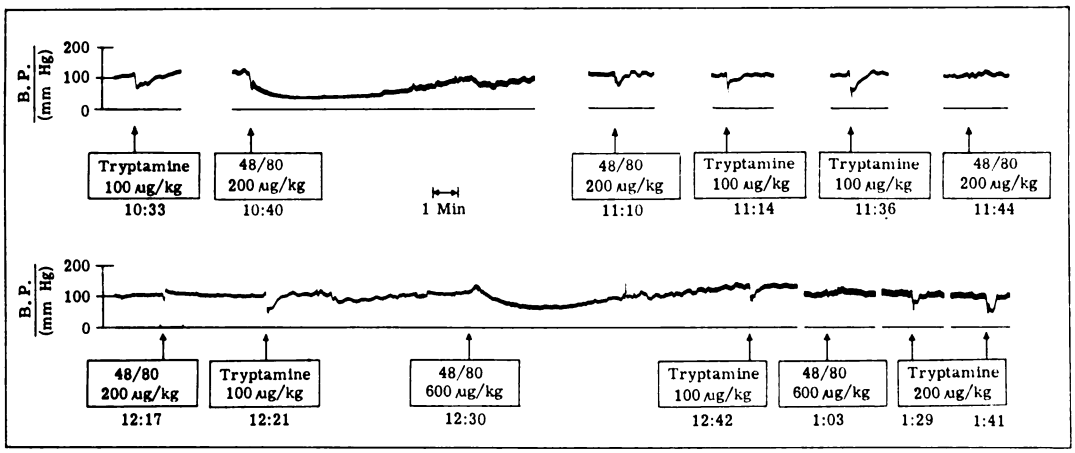

FIG. 3. The effect of 48/80 tachyphylaxis on the arterial blood pressure responses to intravenously administered tryptamine.
Hen, 3.7 kg; intravenous pentobarbital anesthesia.

when tried in 5 animals with cumulative doses as high as 4 mg/kg. A dose of 2 mg/kg completely blocked the depressor effect of isoproterenol.

Cocaine in doses as high as 10 mg/kg did not affect the depressor response to tryptamine in the 2 chickens so tested, but the pressor action of *l*-epinephrine was greatly increased by this dose of cocaine as demonstrated earlier by Vander Brook and Vos (1940).

G. *Perfused leg.* In the study of the peripheral circulation, intraarterial injections of tryptamine (dose range 1 to 100 μg/kg) resulted in an increase of perfusion pressure in 11 chickens. This was also the case when tryptamine was given intravenously, even though the systemic pressure fell (fig. 4). Intraarterial injection of histamine (0.1 to 2.0 μg/kg) in these 11 preparations resulted in a fall of perfusion pressure as did 48/80 (10 μg/kg) in 6 chickens.

H. *Right intraventricular pressures.* The right ventricle was successfully catheterized in 5 chickens and tryptamine was given intravenously 3 to 10 times in each. In all instances, there was a rise in right ventricular pressure preceding by a few heart beats the fall in systemic pressure. A representative experiment is shown in the upper half of figure 5. This increase in the pressure in the right ventricle concomitant with a systemic fall was interpreted as a reflection of an increase in resistance in the pulmonary circulation. The magnitude of the rise in right ventricular pressure increased with increasing doses of tryptamine as did the magnitude of the fall in systemic pressure; doubling of the systolic pressure was a regular finding.

II. PRESSOR ACTION. In untreated chickens, a depressor effect of tryptamine is the usual observation. Treatment with antihistaminics or MAO inhibitors changes this to a pressor action. A detailed analysis of this reversal phenomenon is to be the subject of a separate report; observations on the resulting pressor activity of tryptamine follow.

In this series of experiments, tranylcypromine in doses of 2 to 4 mg/kg was given intravenously to change the tryptamine response to purely pressor.

A. *Adrenergic blocking agents.* Both phenoxybenzamine and tolazoline produced total blockade of *l*-epinephrine in our chicken preparations. Antagonism to norepinephrine was less uniform, but in most chickens, reduction and even blockade of the pressor response to norepinephrine was obtainable with increase in dose. Neither phenoxybenzamine (3 chickens) nor tolazoline (3 chickens) exerted any influence on the pressor response to tryptamine in the chicken. (Since phenoxybenzamine itself unmasks a pressor response to tryptamine, it would not be expected to block this action.)

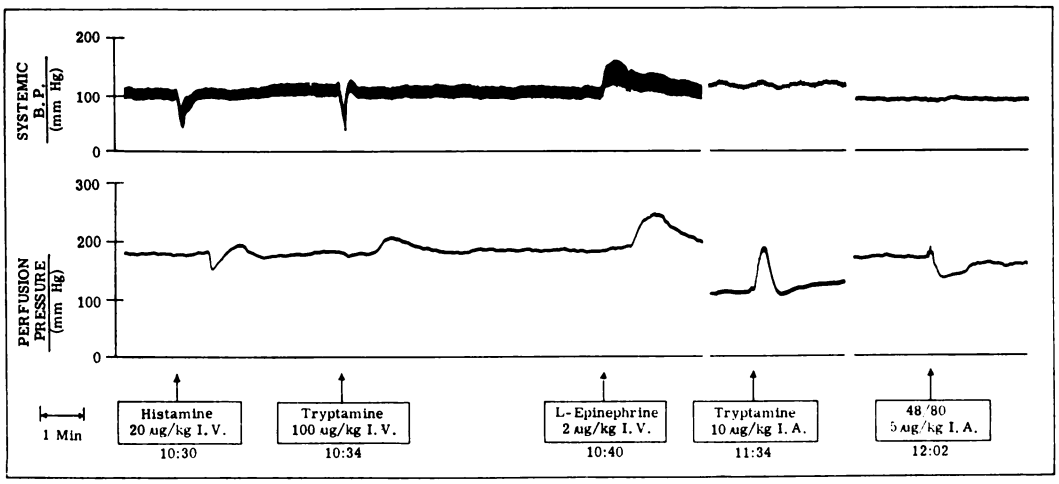

FIG. 4. The effect of intravenous administration of histamine, tryptamine, epinephrine, and 48/80 on arterial blood pressure (top) and perfusion pressure of the leg (bottom) in the anesthetized chicken. The pump system was set at a flow of 20 ml/min. Intraarterial injections were made by needle puncture of the rubber tubing on the output side of the pump. The systemic pulse pressure was electronically dampened in the last two curves. Weight, 3.0 kg; intravenous pentobarbital anesthesia.

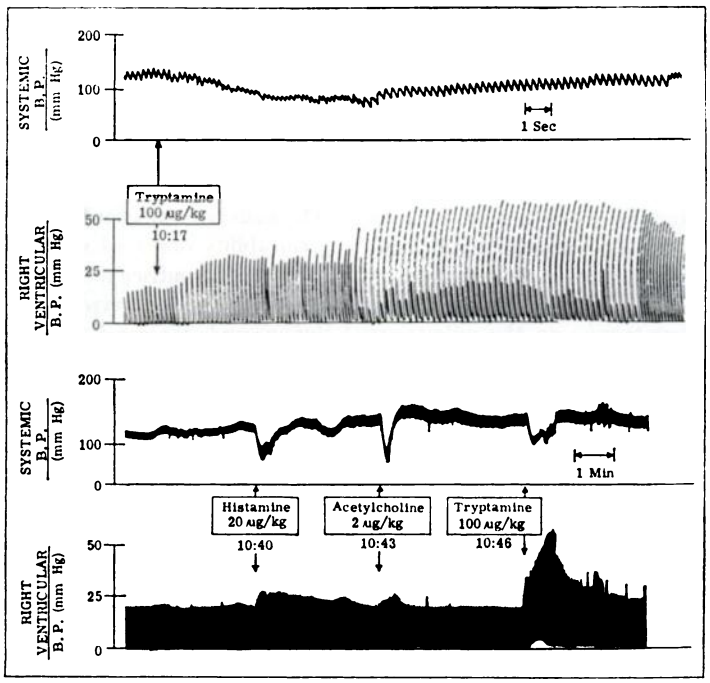

FIG. 5. Top half: expanded time scale showing sequential effects of intravenously administered tryptamine on systemic and right ventricular blood pressures.
Bottom half: same experiment, reduced chart speed; a comparison of the effects of tryptamine with histamine and acetylcholine. Male chicken, 2.0 kg; intravenous pentobarbital anesthesia.

B. *Other characteristics of the pressor effect of tryptamine.* As with the depressor response to tryptamine in the chicken before MAO inhibitor treatment, there also is no tachyphylaxis to the pressor response to tryptamine after treatment with tranylcypromine. Ten consecutive doses of 200 µg/kg tryptamine within a 40-minute period showed no diminution of response. Results in 11 additional chickens were similar.

No cross tachyphylaxis with ephedrine or amphetamine and tryptamine was observed in the chicken in 4 experiments (3 ephedrine, 1 amphetamine).

In 4 experiments, 7 to 10 mg/kg of cocaine were used. The pressor action of tryptamine in the chicken was not affected by cocaine; it was not potentiated as was *l*-epinephrine nor was it antagonized as was ephedrine.

These results indicate that tryptamine cannot be classified with those sympathomimetic amines which act indirectly by the release of endogenous catecholamines.

DISCUSSION. The characteristic response to tryptamine is different from one animal species to another (Page, 1952) and within a species may differ from the response to serotonin not only in quantity, but in kind. For example, Ginzel and Kottegoda (1954) have shown that intracarotid injections of serotonin in the cat result in hypotension; of tryptamine, in a slight rise in blood pressure.

The experiments reported here show tryptamine to be a potent generalized vasoconstrictor in the chicken. The usual fall of systemic blood pressure following the intravenous injection of tryptamine appears to be due primarily to the increase in the resistance in the pulmonary circulation predominating over the increase in resistance in the peripheral or greater circulation. This mechanism of action is indicated by the increase in right ventricular pressure which precedes by some beats the decrease in systemic blood pressure (fig. 5). That an increase in peripheral resistance accompanied the fall in systemic pressure (shown in the constant flow leg perfusion experiments) further contributes to this conclusion. This same mechanism was proposed by Reid (1951) to explain the initial fall of blood pressure resulting from tryptamine in the cat. In the chicken, this would seem to be the dominant

mechanism, as it was in some cats observed by Reid.

The pulmonary circulation of the chicken appears to be especially sensitive to the action of tryptamine in that doses of histamine and acetylcholine, which produce comparable changes in systemic pressure, have much less effect on the pulmonary circulation (lower half of fig. 5). Rose and Lazaro (1958) and Rudolph and Paul (1957) found serotonin to be a more potent pulmonary constrictor than acetylcholine or histamine.

This action of tryptamine on the pulmonary circulation in the chicken is comparable to its action in the cat (Reid, 1951), and the action of serotonin in man and dog (Baldrighi and Ferrari, 1955), cat (Reid, 1952), and dog (Rudolph and Paul, 1957).

The peripheral nature of these vasoconstrictor actions of tryptamine in the chicken was indicated by the experimental results which showed that neither cholinergic nor adrenergic blockade influenced them. Therefore, neither an increase in cholinergic outflow nor a decrease in tonic adrenergic outflow can account for this depressor response to tryptamine and hence, reflex (particularly von Bezold), central and ganglionic mechanisms are not likely. Similar resistance of the vasoconstrictor activity of tryptamine to tolazoline blockade has been shown in other species (Page, 1952; Ginzel and Kottegoda, 1953).

We cannot account for the difference between these results with atropine and those of Buñag and Walaszek (1961) who report atropine to be an effective antagonist to the depressor action of tryptamine in the chicken. Atropine has not otherwise been found to be a good antagonist to the cardiovascular effects of the indole ethylamines (Gaddum, 1953; Reid, 1952; Smith and Smith, 1955; Freyburger *et al.*, 1952).

Buñag and Walaszek (1961, 1962) proposed that tryptamine could induce a release of histamine which might account for its depressor action in the chicken. However, since there was no delay in the depressor response to tryptamine, no tachyphylaxis to the depressor response of tryptamine or cross-tachyphylaxis to the histamine releasing substance 48/80, we believe that histamine release cannot be an important factor in the fall in blood pressure. That 48/80 decreases perfusion pressure when injected intraarterially and that tryptamine increases this perfusion pressure, further indicates that histamine release is not the mechanism of the tryptamine depressor action. In addition, we have observed that 48/80 has very little effect on right ventricular pressures. Lecomte and Beaumariage (1957) reached a similar conclusion regarding the depressor action of serotonin in the chicken on the basis that with serotonin, there was not the cutaneous vasodilation or increase in capillary permeability observed with the histamine effect.

Although ventricular depression in the chicken by serotonin and tryptamine was reported by Buñag and Walaszek (1961), no relationship could be established between the degree of ventricular depression and the magnitude of the blood pressure fall, or between antagonism of pressor effects and cardiac effects. The fall in systemic pressure in our chickens was not usually accompanied by any significant bradycardia as can be seen in figure 5, although in this instance there was a fleeting decrease in heart rate during the recovery phase of the blood pressure.

In the dog, serotonin acts as a cardiac stimulant rather than a depressant (Page, 1958). We do not think it likely that an action of tryptamine on the chicken heart contributes significantly to the depressor effect, but further work would be necessary to eliminate completely this possibility.

The serotonin-induced fall in blood pressure has been attributed to peripheral inhibition of neurogenic vasoconstrictor tone (Page, 1958; McCubbin *et al.*, 1962). This would not seem to be an operating mechanism for the tryptamine fall in the chicken because tryptamine depressor effects are unaffected in the chicken treated with tolazoline to the point where there is no response to *l*-epinephrine or norepinephrine. The similarity between our blood pressure tracings for tryptamine in the chicken and those shown for serotonin in the rat (Salmoiraghi and Page, 1956) and in the cat and rabbit (Schneider and Yonkman, 1954) suggests that pulmonary vasoconstriction overcoming the peripheral vasoconstriction may be an important factor in the blood pressure fall in those species also.

The distinctiveness of tryptamine receptors has been well defined in several reports; for guinea-pig ileum (Gaddum, 1953) and for dog vasoconstrictor responses (Woolley and Shaw, 1957). Page (1952) has previously written "it appears unlikely that the conventional cholinergic-adrenergic mechanism describes the action of serotonin." This statement applies to the

vasoconstrictive action of tryptamine in the chicken. Adrenergic and cholinergic blockade are without effect, cocaine neither potentiates nor antagonizes tryptamine vasoconstriction, there is no tachyphylaxis to this action or cross-tachyphylaxis with ephedrine. These results make it clear that tryptamine does not fit into the classification of cardiovascular active sympathomimetic amines set forth by Burn.

SUMMARY

The depressor effect of tryptamine in the chicken was shown to be accompanied by a rise in peripheral resistance and by a rise in right ventricular pressure. The systemic fall in pressure appeared to be the result of an acute pulmonary hypertension. It was not affected by cholinergic blockade with atropine or methylatropine, or by adrenergic blockade with tolazoline or dichloroisoproterenol, and it was found to be reversed by the MAO inhibitor, tranylcypromine.

That this vasoconstrictor action of tryptamine is not related to cholinergic or adrenergic mechanisms is indicative of the specificity of tryptamine receptors in the chicken cardiovascular system.

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