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The Septal-Hippocampal Cholinergic Pathway: Role in Antagonism of Pentobarbital Anesthesia and Regulation by Various Afferents

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ABSTRACT

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Earlier studies have demonstrated that pentobarbital reduces the turnover rate of acetylcholine (ACh) in hippocampus and that this effect may be mediated *via* the septal-hippocampal cholinergic pathway. Moreover, the narcosis associated with the administration of pentobarbital may be reversed by intraseptal injection of such chemically diverse compounds as bicuculline, a potent γ-aminobutyric acid antagonist; thyrotropin-releasing hormone, a neuroactive tripeptide; and kainic acid, a rigid analog of glutamate. To determine whether or not these three compounds modulate the metabolism of ACh in hippocampus, they have been injected intraseptally in pentobarbital-pretreated rats and the turnover rate of ACh has been determined by gas chromatography-mass fragmentography. Pentobarbital produces a dose-dependent decrease in the turnover rate of ACh in cortex and hippocampus but not in striatum. The effect appears to be maximum at 30 min and returns to normal within 15 min of recovering the righting reflex. Slow local infusion of either bicuculline or thyrotropin-releasing hormone into the septum reverses the pentobarbital-induced narcosis and antagonizes the pentobarbital-induced decrease in the hippocampal turnover rate of ACh. Administration of kainic acid into the lateral, but not the medial, septum reduces specifically the glutamic acid decarboxylase activity in the ipsilateral septum without altering the choline acetyltransferase activity or the turnover rate of ACh in the hippocampus. Moreover, kainic acid injected into the lateral septum antagonizes the pentobarbital narcosis and reverses the pentobarbital-induced decrease in the ACh turnover rate in the ipsilateral hippocampus, but not in the contralateral hippocampus. It appears that all three compounds antagonize the pentobarbital-induced decrease in hippocampal ACh turnover rate and the pentobarbital narcosis by modulating neurons in the lateral septum, presumably through an action on the GABAergic interneurons.

The septal-hippocampal cholinergic pathway can be modulated by various neuronal afferents (Moroni *et al.*, 1977; Robinson *et al.*, 1979a,b; Wood *et al.*, 1979). The activity in some of these afferents may change during pentobarbital-induced anesthesia (Atweh and Kuhar, 1976; Rommelspacher *et al.*, 1974). This change may be associated with modifications of the hippocampal TR_{ACh}. In fact, pentobarbital has been shown to reduce the turnover of ACh in the nerve terminals of the septal-hippocampal pathway (Moroni *et al.*, 1978). This reduction may be reversed by electrical stimulation of the medial septum (Moroni *et al.*, 1978) which contains the cell bodies of the septal-hippocampal cholinergic pathway (Dudar, 1975; Linch *et al.*, 1977; Mosko *et al.*, 1973; Sethy *et al.*, 1973; Shute and Lewis, 1961). Furthermore, intraseptal administration of TRH (Kalivas and Horita, 1979, 1980), of bicuculline, a GABA antagonist (Brunello and Cheney, 1980) and of kainic acid, a rigid analog of glutamate (Brunello and Cheney, 1980) reverse pentobarbital anesthesia. Therefore, the experiments reported here have been performed 1) to assess whether bicuculline, TRH or kainic acid counteract the depression of the ACh metabolism in the septal-hippocampal neurons elicited by the administration of pentobarbital and 2) to detect the site of action and to elucidate the mechanisms whereby these three compounds modulate septal-hippocampal cholinergic neurons.

A direct correlation between changes in the TR_{ACh} and in the activity of hippocampal cholinergic neurons has been shown after electrical stimulation of the septum or transection of the fimbria (Moroni *et al.*, 1978). These two procedures cause either an increase or a decrease of the turnover rate of hippocampal ACh, respectively, indicating that the TR_{ACh} in the hippocampus increases or decreases proportionally to the activity of the septal cholinergic neurons. Because of the correlation mentioned above (Moroni *et al.*, 1978), the TR_{ACh} has been taken as an index of a drug-induced change in ACh metabolism and

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ABBREVIATIONS: TR_{ACh}, turnover rate of acetylcholine; ACh, acetylcholine; TRH, thyrotropin-releasing hormone; GABA, γ-aminobutyric acid; k_{ACh}, rate constant for acetylcholine efflux; ChAT, choline acetyltransferase; GAD, glutamic acid decarboxylase.

inferences have been made concerning the activity of cholinergic neurons.

Methods

Male Sprague-Dawley rats (Zivic-Miller, Allison Park, PA) weighing 140 to 170 g were used throughout the experiments. In some studies, sodium pentobarbital (35 mg/kg) (Lannett Co., Philadelphia, PA) was administered i.p. to rats and 21 min later bicuculline methiodide (Pierce Chemicals, Rockford, IL), TRH (Beckman Instruments, Inc., Palo Alto, CA), kainic acid (Sigma Chemical Company, St. Louis, MO) or phosphate buffer (160 mM; pH 7.4) was injected into the medial septum (A, 7.8; L, 0.0; V, −0.3) (Konig and Klippel, 1963). The compounds were dissolved in the phosphate buffer and a total volume of 1 μl was injected over a 9-min period. During the last 9 min, the animals were infused through the lateral tail vein with phosphoryl $(CD_3)_3$ choline (135 μmol/kg) (Zsilla *et al.*, 1977). In other studies, under pentobarbital anesthesia (35 mg/kg i.p.), the rats were stereotaxically implanted with stainless-steel guide cannulas (22-gauge, Plastic Products Co., Roanoke, VA) placed in the medial septum using the coordinates mentioned above. Bicuculline (35 μg) or phosphate buffer was injected 3 days after surgery using a gear-driven syringe microburet (Micro-Metric Instrument Co., Cleveland, OH) connected by PE 20 polyethylene tubing with an internal cannula (28-gauge) extending 1.0 mm beyond the tip of the guide cannula. The volume of the injection was 1 μl which was delivered over a period of 30 sec. At the appropriate time, the rats were killed by a beam of microwave irradiation focussed on the head (Guidotti *et al.*, 1974). The brain areas, frontal cortex, parietal cortex, striatum and hippocampus were dissected and the tissues were analyzed for ACh, choline and their deuterated analogs by gas chromatography-mass fragmentography (Zsilla *et al.*, 1977). From the percentage of incorporation of deuterium label in ACh and choline, the fractional k_{ACh} was calculated and the TR_{ACh} was obtained by multiplying the k_{ACh} by the steady-state concentration of ACh (Racagni *et al.*, 1974). Because the data obtained from analysis of frontal cortex and parietal cortex were not significantly different from each other, they were combined for presentation in the figures. In other studies, rats were anesthetized (pentobarbital, 35 mg/kg i.p.) and injected with kainic acid or phosphate buffer either in the medial septum, using the coordinates mentioned above, or lateral septum (A, 7.8; L, 0.5; V, −0.3) (König and Klippel, 1963). Kainic acid was injected over a period of 5 min. After 7 days, the animals were killed by decapitation and the brains were rapidly removed and dissected to obtain the septum and hippocampus. The brain parts were kept frozen until the enzymatic assay for ChAT (Kobayashi *et al.*, 1975) and GAD (MacDonnel and Greengard, 1975). The data for all experiments were analyzed statistically using the multiple comparison test of Dunnett (Winer, 1971). Doses of drug administered refer to the salt.

Results

Thirty minutes after the i.p. administration of various doses of pentobarbital (fig. 1), there was a dose-dependent decrease in the TR_{ACh} in hippocampus and cortex. A dose of 18 mg/kg of pentobarbital failed to change the TR_{ACh} in either structure, whereas 65 mg/kg caused an 83% reduction in TR_{ACh} in these two brain areas. The TR_{ACh} in striatum was unaltered by any of the doses administered. Furthermore, 30 min after administration of the various doses studied, the concentrations of ACh and choline were not affected in cortex, striatum or hippocampus (data not shown). The concentration and TR_{ACh} in striatum, hippocampus and cortex were measured at various times after the i.p. injection of pentobarbital (35 mg/kg) as shown in figure 2. The pentobarbital sleeping time after this dose was 80 ± 8.2 min as indicated by the shaded areas in figure 2. In the striatum, neither the ACh content nor the TR_{ACh} was altered at any time after this dose of pentobarbital. As also shown in figure 1, 30 min after administration of 35 mg/kg of pentobarbital, the TR_{ACh} in hippocampus and cortex was significantly reduced without a concomitant change in the ACh concentration. After 60 min, however, the situation changed. There was a slight increase in ACh content in both cortex and hippocampus which was significant when compared to control animals, but not when compared to animals treated with pentobarbital for 30 min. The changes in TR_{ACh} did not parallel the ACh increase. In the cortex, the TR_{ACh} was not significantly different from controls, whereas in the hippocampus the TR_{ACh} was still significantly reduced. Fifteen minutes after recovery of the righting reflex (95 ± 9.7 min), the concentration of ACh and the TR_{ACh} were not significantly different from control values in these three brain areas (fig. 2).

Intraseptal injection of bicuculline in pentobarbital-treated animals. Animals were implanted with a chronic cannula in the medial septum under anesthesia. Three days later, the TR_{ACh} in cortex and hippocampus was determined

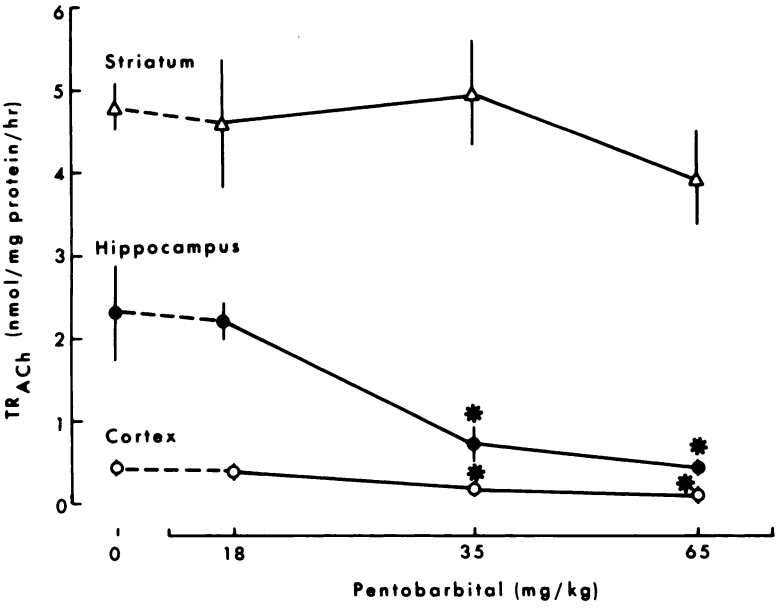

Fig. 1. Acetylcholine turnover rate in rat striatum (△), hippocampus (●) and cortex (○) 30 min after i.p. administration of sodium pentobarbital. Controls were injected with an equal volume of physiologic saline. Asterisk indicates $P < .05$ compared to control.

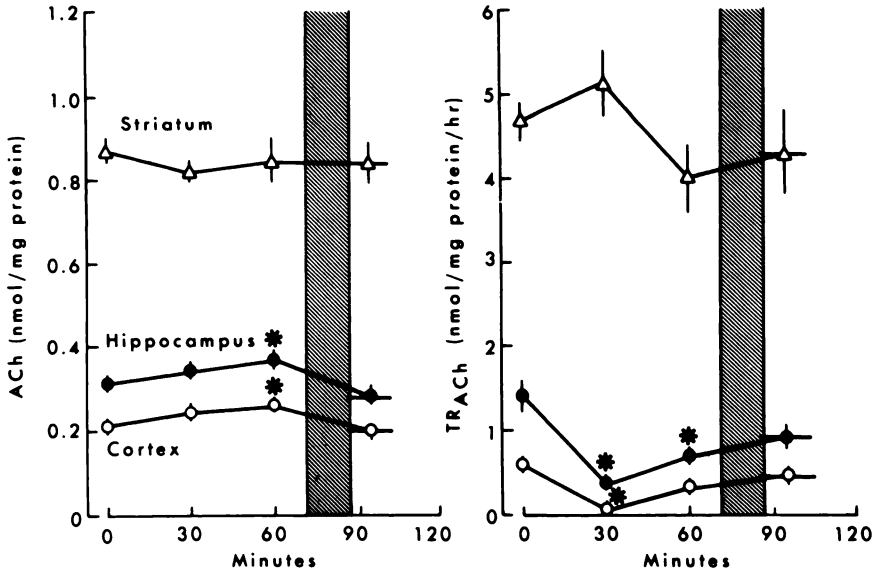

Fig. 2. Acetylcholine concentration and turnover rate in rat striatum (△), hippocampus (●) and cortex (○) at various times after i.p. administration of sodium pentobarbital (35 mg/kg). Control rats were injected with an equal volume of physiologic saline. Shaded area represents the sleeping time (80 ± 8.2 min) of rats treated with this dose of pentobarbital. Asterisk indicates a significant difference ($P < .05$) compared to zero time. Each data point represents mean ± S.E.M. of six to seven animals.

after the administration of pentobarbital (35 mg/kg i.p., 40 min) and/or bicuculline. Bicuculline (35 μg) was dissolved in 1 μl and infused into the medial septum over a 30-sec period 10 min after the injection of pentobarbital. The rats were killed 30 min later by focussed microwave irradiation.

After the 30-sec intraseptal injection of bicuculline, the rats rapidly regained their righting reflex. In addition, bicuculline reversed the effects of pentobarbital on ACh metabolism, as demonstrated in table 1, bringing the values for k_{ACh} and TR_{ACh} back to the control values in hippocampus and, surprisingly, in cortex. When administered alone, however, bicuculline had no effect on the concentration or TR_{ACh} in either cortex or hippocampus.

In order to minimize the bicuculline-induced reversal of the pentobarbital action in cortex which may have been due to diffusion or backflow along the cannula, bicuculline (35 μg in 1 μl) was infused slowly into the medial septum during the 9-min period immediately preceding the brain irradiation with microwaves. The slow infusion of bicuculline into the medial septum caused a specific reversal of the pentobarbital-induced decrease of the hippocampal k_{ACh} and TR_{ACh} (table 2) to 60% of control values without changing the reduced cortical TR_{ACh} caused by the barbiturates in cortex (table 2). The ACh content remained unaffected in both areas.

Intraseptal injection of TRH in pentobarbital-treated animals. TRH antagonized pentobarbital narcosis when injected into a number of neuronal sites at a dose of 0.5 μg (Kalivas and Horita, 1979). The septal region was the most sensitive, responding at one-tenth of this dose. A dose of 0.5 μg of TRH reduced the sleeping time by 50% (Kalivas and Horita, 1979). A slow infusion (9 min) of 0.5 μg in 1 μl immediately before killing the rats by microwave irradiation not only reversed the pentobarbital-induced narcosis but, as shown in table 3, also reversed the pentobarbital-induced decrease in the k_{ACh} and the TR_{ACh}.

Effect of kainic acid into septum of pentobarbital animals. Kainic acid (1 μg) was injected over a period of 9 min into the left lateral septum beginning 21 min after the i.p. injection of pentobarbital (35 mg/kg), and the rats were killed at the end of the infusion period. This dose of kainic acid antagonized the pentobarbital-induced decrease in the TR_{ACh} in the ipsilateral (left) hippocampus (fig. 3) and partially reversed the barbiturate narcosis. The effect was specific inasmuch as kainic acid did not reverse the action in the contralateral (right) hippocampus or in either side of the frontal cortex (fig. 3).

TABLE 1

TR_{ACh} in cortex and hippocampus after pentobarbital (35 mg/kg i.p., 40 min) and bicuculline (30 μg, 30 sec septal infusion, 30 min)

Data are expressed as mean ± S.E.M. of six to seven determinations. Control rats were injected with an equal volume of the appropriate vehicle.

	ACh	k_{ACh}	TR_{ACh}
	nmol/mg protein	*hr^{-1}*	*nmol/mg protein/hr*
Cortex			
Control	0.18 ± 0.010	3.5 ± 0.19	0.62 ± 0.034
Pentobarbital	0.22 ± 0.012	1.1 ± 0.23*	0.19 ± 0.045*
Bicuculline	0.22 ± 0.020	3.5 ± 0.60	0.71 ± 0.11
Pentobarbital + bicuculline	0.22 ± 0.020	2.9 ± 0.27	0.59 ± 0.033
Hippocampus			
Control	0.29 ± 0.011	5.2 ± 0.75	1.5 ± 0.30
Pentobarbital	0.33 ± 0.042	1.4 ± 0.16*	0.45 ± 0.071*
Bicuculline	0.31 ± 0.028	4.8 ± 1.2	1.5 ± 0.11
Pentobarbital + bicuculline	0.30 ± 0.017	6.0 ± 1.1	1.8 ± 0.39

* $P < .05$.

TABLE 2

ACh turnover rate in hippocampus and cortex of animals pretreated with pentobarbital (35 mg/kg i.p., 30 min) and infused with bicuculline (35 μg, 9 min) into the medial septum

Infusion of bicuculline was initiated 21 min after injection of pentobarbital and the animals were microwaved 9 min later. Data are expressed as mean ± S.E.M. of seven animals. Control rats were injected with an equal volume of the appropriate vehicle.

	ACh	k_{ACh}	TR_{ACh}
	nmol/mg protein	*hr^{-1}*	*nmol/mg protein/hr*
Cortex			
Control	0.19 ± 0.010	3.7 ± 0.15	0.71 ± 0.025
Pentobarbital	0.18 ± 0.0010	1.2 ± 0.30*	0.18 ± 0.033*
Pentobarbital + bicuculline	0.19 ± 0.020	1.3 ± 0.28*	0.25 ± 0.042*
Hippocampus			
Control	0.33 ± 0.027	5.1 ± 0.71	1.7 ± 0.15
Pentobarbital	0.35 ± 0.097	0.90 ± 0.33*	0.32 ± 0.046*
Pentobarbital + bicuculline	0.32 ± 0.027	3.1 ± 0.40*,**	1.0 ± 0.089*,**

* $P < .01$ *vs.* control; ** $P < .01$ *vs.* pentobarbital-treated animals.

In order to investigate the location and the type of neurons that participate in the regulation of cholinergic septal neurons, kainic acid was injected into either the medial septum, the left lateral septum or bilaterally. The rats were sacrificed after 7 days. After injection of kainic acid into the medial septum at a dose of 0.5 μg, neither the septal GAD activity nor the hippocampal ChAT activity changed (table 4). Moreover, the hippocampal TR_{ACh} was unaffected. However, the same amount of kainic acid injected into the left lateral septum reduced GAD activity in the ipsilateral (left) septum by 36%, compared to the contralateral (right) septum. No significant changes in ChAT activity could be detected in the ipsilateral (treated side) hippocampus compared to the contralateral (control side) hippocampus (table 4). In the turnover studies, 7 days after administration of kainic acid into the left lateral septum, there was no modification of the TR_{ACh} in the hippocampus (table 4). Furthermore, neither unilaterally nor bilaterally injected kainic acid into the lateral septi modified the ability of pentobarbital to reduce the TR_{ACh} in hippocampus (table 5) nor the pentobarbital narcosis.

Discussion

It has been established that the administration of pentobarbital decreases the impulse flow in many central neurons (Bradley and Dray, 1973). In cholinergic neurons, anesthetic doses of pentobarbital inhibit TR_{ACh} estimated by the rate of ACh decline after intraventricular injection of hemicholinium-3 (Domino and Wilson, 1972). Anesthetic doses of pentobarbital also inhibit the release of ACh from the cerebral cortex (Beani *et al.*, 1968; Mitchell, 1963) and reduce the choline uptake in cerebral cortex (Atweh *et al.*, 1975; Simon *et al.*, 1976; Trabucchi *et al.*, 1975), hippocampus (Atweh *et al.*, 1975; Simon *et al.*, 1976) and hypothalamus (Simon *et al.*, 1976), but not in stria-

TABLE 3

ACh turnover rate in hippocampus of pentobarbital-pretreated animals (35 mg/kg i.p., 30 min) infused with TRH (0.5 μg, 9 min) into the medial septum

Infusion of TRH administration was initiated 21 min after injection of pentobarbital and the animals were microwaved 9 min later. Data are expressed as mean ± S.E.M. of seven animals. Control rats were injected with an equal volume of the appropriate vehicle.

	ACh	k_{ACh}	TR_{ACh}
	nmol/mg protein	*hr^{-1}*	*nmol/mg protein/hr*
Control	0.33 ± 0.017	4.9 ± 0.033	1.6 ± 0.14
Pentobarbital	0.32 ± 0.018	0.98 ± 0.043*	0.35 ± 0.050*
Pentobarbital + TRH	0.34 ± 0.040	3.5 ± 0.19**	1.2 ± 0.10**

* $P < .01$ *vs.* control; ** $P < .01$ *vs.* pentobarbital-treated animals.

TABLE 4

Effect of kainic acid (0.5 μg, 7 days) injected into medial septum or left lateral septum

Data are expressed as mean ± S.E.M. of at least seven animals. Control rats were injected with an equal volume of the appropriate vehicle.

	GAD Activity in the Septum	ChAT Activity in Hippocampus	ACh Turnover Rate in Hippocampus
	nmol [^{14}C]CO_2 formed/mg protein/min	*nmol [^{14}C]ACh formed/mg protein/min*	*nmol/mg protein/hr*
Medial Septum			
Control rats	12 ± 0.85	0.38 ± 0.01	1.4 ± 0.22
Treated rats	12 ± 0.92	0.37 ± 0.02	1.3 ± 0.19
Left Lateral Septum			
Control side	12 ± 0.47	0.38 ± 0.04	1.8 ± 0.24
Ipsilateral side	7.4 ± 0.34*	0.38 ± 0.03	1.7 ± 0.19

* $P < .02$ *vs.* contralateral side.

TABLE 5

Turnover rate of ACh in hippocampus after administration of pentobarbital (35 mg/kg i.p., 30 min) to rats injected with kainic acid in lateral septum bilaterally (1 μg each side, 7 days) or unilaterally (1 μg left side, 7 days)

Data are expressed as mean ± S.E.M. of seven animals. Control rats were injected with an equal volume of the appropriate vehicle.

	TR_{ACh}		
	Right side	Left side	Combined
		nmol/mg protein/hr	
Sham			1.8 ± 0.095
Bilateral kainic acid			1.9 ± 0.14
Unilateral kainic acid (left lateral septum)	1.8 ± 0.26	1.9 ± 0.13	
Sham + pentobarbital			0.80 ± 0.18*
Bilateral kainic acid + pentobarbital			0.46 ± 0.22*
Unilateral kainic acid (left lateral septum) + pentobarbital	0.70 ± 0.093*	0.75 ± 0.18*	

* $P < .01$.

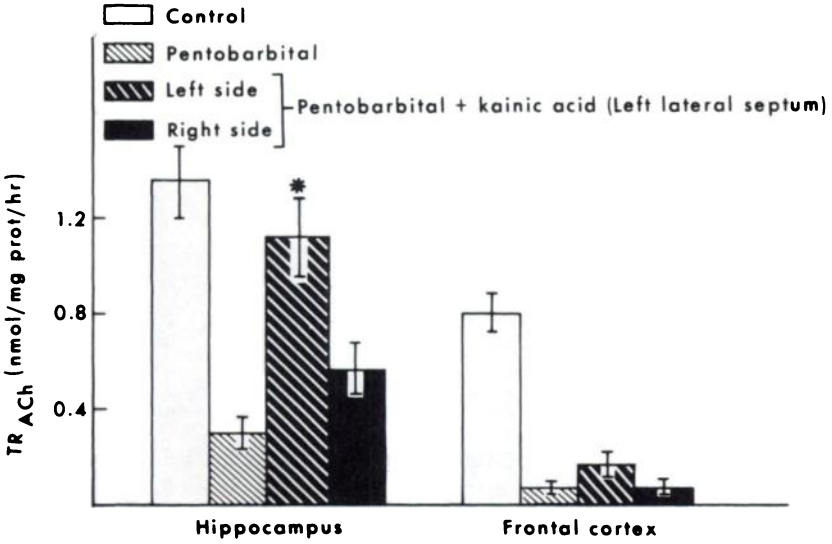

Fig. 3. Effect of kainic acid (1 μg; 9 min) injected into the left lateral septum on the TR_{ACh} in sodium pentobarbital (35 mg/kg i.p., 30 min)-treated animals. Control rats were injected with an equal volume of the appropriate vehicle. Asterisk indicates $P < .01$ compared to pentobarbital-treated rats. Data are expressed as mean ± S.E.M. of seven animals.

tum (Atweh *et al.*, 1975; Trabucchi *et al.*, 1975). As shown in figures 1 and 2, after pentobarbital treatment there is a tremendous decrease in ACh turnover rate in cerebral cortex as well as in the hippocampus, whereas there is no significant change in the striatum. The decrease in ACh turnover rate is due to a lower fractional rate constant for ACh efflux and not to a modification of ACh steady-state concentrations. Higher doses and/or longer periods of administration of pentobarbital increase the steady-state content of ACh, but this increase is not sufficient to compensate for the reduced k_{ACh} and the TR_{ACh} remains diminished (Moroni *et al.*, 1978; Trabucchi *et al.*, 1975).

The observations that pentobarbital profoundly decreases the TR_{ACh} in the cerebral cortex and hippocampus but not the striatum suggests that barbiturates decrease ACh metabolism, not by a direct action on enzymes operative in the regulation of ACh but by an indirect, perhaps, transsynaptic mechanism which decreases the firing rate of septal hippocampal neurons. Such an hypothesis is in agreement with the view (Atweh and Kuhar, 1976) that pentobarbital decreases the activity of the cholinergic septal-hippocampal neurons. Some of the effects of pentobarbital in the hippocampus (Atweh and Kuhar, 1976; Rommelspacher and Kuhar, 1974) can be reversed by electrical stimulation of the cell bodies of cholinergic neurons which are located in the medial septum. The narcosis induced by pentobarbital can be antagonized by intraseptal injections of bicuculline (Brunello and Cheney, 1980), TRH (Kalivas and Horita, 1979, 1980) or kainic acid (Brunello and Cheney, 1980). The observation that such chemically diverse compounds as bicuculline, TRH and kainic acid reversed the pentobarbital-induced narcosis prompted the study of whether or not these compounds modulate the metabolism of ACh in the septal-hippocampal pathway after administration of pentobarbital.

The cell bodies of this well-defined cholinergic pathway (Lewis and Shute, 1967) are located in the medial septal nucleus and in the nucleus of the diagonal band (Mosko *et al.*, 1973). Their axons project *via* the fimbria (Raisman, 1966) to the pyramidal cells located in the stratum oriens and the granular cells located in the dentate gyrus. It has been postulated that this cholinergic pathway may be regulated by two major intrinsic neuronal groups (McLennan and Miller, 1974a,b; DeFrance *et al.*, 1975; McLennan and Miller, 1976): 1) inhibitory interneurons located in the medial septum and 2) a recurrent collateral loop acting on inhibitory neurons located in the lateral septum and projecting to the medial septum. Although the chemical nature of these neuronal groups has not been identified, it has been shown that dopamine (Cheney *et al.*, 1978, 1979; Robinson *et al.*, 1979b) and β-endorphin (Wood *et al.*, 1979) neurons terminating in the lateral septum appear to modulate the activity of the cholinergic septal-hippocampal neurons originating in the medial septum *via* inhibitory GABAergic neurons, which perhaps are located in the lateral septum and project to the medial septum.

In the experiments reported here, blockade of GABA receptors by a slow infusion of bicuculline into the medial septum of rats pretreated with pentobarbital antagonizes the barbiturate narcosis and reverses the decreased TR_{ACh} in hippocampus (table 2). Thus, slow septal infusion of bicuculline causes a selective reversal of the decrease of ACh turnover in hippocampus. The data from table 1 suggest that a rapid injection of bicuculline into the septum causes a more general reversal of the pentobarbital-induced decrease in the TR_{ACh} in cortex as well as hippocampus, perhaps by diffusion or bulk flow into the cerebral ventricles. The specific antagonism of the reduction of TR_{ACh} in hippocampus after the slow localized infusion of bicuculline into the medial septum would suggest that GABAergic neurons interconnect the lateral and medial septa and that blockade of septal GABAergic receptors causes the same effect as stimulation of the neurons of the cholinergic septal-hippocampal pathway. Thus, if monosynaptic GABAergic interneurons interconnected the lateral and medial septum then pentobarbital might be expected to increase the GABAergic activity in the septum. However, the turnover rate of GABA in the septum after administration of pentobarbital fails to increase (Dr. Aurora Revuelta, unpublished data), raising the possibility that the special arrangement of the GABA neurons interconnecting the lateral and medial septum is more complex.

It is tempting to speculate that TRH may reverse the barbiturate narcosis and restore the cholinergic activity *via* the septal GABAergic interneurons. In addition to its endocrinological effects, TRH exerts profound effects on the central nervous system (Horita *et al.*, 1979; Nemeroff *et al.*, 1978). The unique and nonuniform pattern of distribution of TRH throughout the regions of the brain (Brownstein *et al.*, 1974; Hökfelt *et al.*, 1975) and its localization in nerve terminals (Winokur *et al.*, 1977) indicates that it may function as a neuromodulator. TRH is more highly concentrated in the lateral septum than in the medial septum (Brownstein *et al.*, 1974; Hökfelt *et al.*, 1975) and microinjection of TRH into specific brain loci have implicated the lateral septum as an important site of action of TRH antagonism of pentobarbital narcosis (Kalivas and Horita, 1979). Moreover, cholinergic activation has been proposed to mediate the antagonism of pentobarbital narcosis by TRH (Kalivas and Horita, 1980). After slow localized infusion of TRH into the lateral septum of pentobarbital-treated animals, there is an increase in the hippocampal ACh turnover rate compared to the value found in pentobarbital-treated animals (table 3). This would suggest that TRH neurons located in the lateral septum exert a neuromodulatory role on the neurons of the cholinergic septal-hippocampal pathway *via* the septal GABAergic interneurons.

Besides the intrinsic inhibitory GABAergic control, the lateral septum receives important collaterals from the pyramidal cells in field CA_3 of the hippocampus that pass through the fimbria (DeOlmos and Heimer, 1977; Raisman, 1966; Swanson and Cowan, 1977). Recent evidence suggests that these axons use glutamate as a neurotransmitter (Fonnum and Walaas, 1978; Storm-Mathisen and Woxen-Opsahl, 1978). Kainic acid, the potent rigid analog of glutamate, has been used to identify the nature of the cells involved in the feedback regulation of the septal neurons.

Seven days after the injection of kainic acid into the medial septum, the GAD activity in the septum itself or the ChAT activity in the hippocampus remains unchanged (table 4). Furthermore, the ACh turnover rate in the hippocampus is not altered. Since the cells in the medial septum appear to be resistant to kainic acid, probably they are not innervated by glutamatergic fibers (Köhler *et al.*, 1978). Conversely, the same amount of kainic acid injected into the lateral septum reduces specifically the GAD activity in the ipsilateral septum, whereas no significant change in ChAT activity or ACh turnover rate can be detected in the hippocampus after this lesion (table 4). This would be consistent with the possibility that GABAergic neurons in the lateral septum are innervated by glutamatergic nerve terminals.

The injection of kainic acid into the lateral septum of animals

pretreated with pentobarbital can reverse the behavioral effect of barbiturate and antagonize the pentobarbital-induced decrease of the ACh turnover rate in the ipsilateral hippocampus. The TR_{ACh} in the contralateral hippocampus and in either side of the frontal cortex is not significantly different from the value found in sham-injected pentobarbital-treated animals. These data would suggest that stimulation of glutamatergic receptors in the lateral septum exerts an excitatory action on the cholinergic pathway. Most likely the cells in the lateral septum which receive the glutamatergic afferents from the hippocampus are inhibitory GABAergic interneurons whose cell bodies are located in the lateral septum. However, the loss of the GABAergic interneurons in the lateral septum as evidenced by the reduction of GAD activity 7 days after injection of kainic acid into the lateral septum does not inhibit the action of pentobarbital to reduce the TR_{ACh} in hippocampus nor does it inhibit the pentobarbital-induced narcosis. These data demonstrate that, although pentobarbital narcosis and hippocampal TR_{ACh} may be modulated *via* GABAergic septal interneurons, these neurons are not obligatory components of the mechanism whereby pentobarbital induces anesthesia.

In conclusion, it can be postulated that 1) the septum may be involved in the antagonism of pentobarbital narcosis and that 2) the septal-hippocampal cholinergic pathway may be involved in the reversal of pentobarbital-induced depression of ACh turnover rates in the hippocampus. Whether there is a causal relationship between the two events will require further investigation. Both the pentobarbital narcosis and the reduced ACh turnover rate in the hippocampus can be reversed by blockade of GABA receptors in the medial septum and by stimulation of glutamatergic receptors and TRH receptors located in the lateral septum. Further studies need to be done in order to identify the recipient cells for TRH action in the lateral septum and to determine whether each compound is acting through a unique set of GABAergic interneurons.

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