
Hippocampal rhythmic slow activity (RSA) in the cat after intraseptal injections of muscarinic cholinolytics

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Abstract. Influence of intraseptal injections of cholinolytics on hippocampal rhythmic slow activity (RSA) was investigated in cats. Results of the first experiment have shown that neither atropine (At) nor scopolamine (Sc) injected into the medial septum in doses of 20 µg prevented the induction of RSA by a cholinergic (carbachol - CCh) stimulation of the anterior hypothalamus as well as by electrical stimulation of posterior hypothalamus or periaqueductal grey substance (PAG), and were only partially effective in prevention of the spontaneous RSA. RSA did not appear spontaneously and could not be induced by electrical brain stimulation after intraperitoneal injections of At (2.0 mg/kg) or Sc (0.2 mg/kg). Results of the second experiment have shown that intraseptal injections of At (5.0 or 10.0 µg) are also ineffective in prevention of RSA induction by CCh (2.5 µg) injected into the same septal locus. The data suggest that the medial septum cannot be regarded as the common muscarinic link within the RSA generating system of the cat.

Key words: hippocampal rhythmic slow activity (RSA), septum, atropine, scopolamine, carbachol, electrical brain stimulation

INTRODUCTION

One of the effects of systemic injection of muscarinic cholinolytics in the cat is a total disappearance of the rhythmic slow activity (RSA) in the hippocampal EEG (Tori and Wikler 1966, Gralewicz 1981). It has been postulated (Bennett et al. 1978) that in this species, contrary to the rabbit and the rat (Bland 1986), exists only one RSA generating mechanism, which is controlled by the cholinergic (muscarinic) system.

There are some data suggesting that at least a part of the cholinergic neurones mediating RSA generation in the cat are located within the hypothalamus (Friedman and Wikler 1970).

In some earlier studies (Gralewicz 1983) we found that in the cat microinjections of atropine sulphate (At) into the hypothalamus did not prevent the induction of RSA by a following injection of carbachol (CCh), a cholinergic agonist, into the same locus. Injection of a nicotinic blocker, hexamethonium (Hx) was also ineffective, but when both, At and Hx, were injected jointly into the hypothalamus the following injection of CCh produced no RSA in the majority of subjects, and in all of them when At alone was administered intraperitoneally. The data suggested that, (1) in the cat hypothalamus not only muscarinic but also nicotinic elements participate in the induction of RSA, and (2) there exists probably a common muscarinic link within the RSA generating system.

Numerous data leave no doubt that in the RSA generating system the neurones of the medial septum play a crucial role (Petsche et al. 1962, Stumpf et al. 1962, Parmeggiani 1967, Wilson et al. 1976, Rawlins et al. 1979, Sainsbury and Bland 1980, Mitchell et al. 1982, Brazhnik and Vinogradova 1986). It has been shown that a direct intraseptal application of muscarinic blockers may eliminate the RSA accompanying the performance of an instrumental response in cats (Bennett 1975) as well as type 2 RSA in urethanized rats (Stewart and Fox 1989). These data allow one to assume that the suggested common muscarinic link of the RSA generating system may be located within the medial

septum. The purpose of the presented two experiments was to check this supposition.

EXPERIMENT I

According to Bennett (1975), a direct infusion of scopolamine (Sc) into the medial septum can block temporarily the RSA accompanying the performance of an instrumental responses in the cat. If the medial septum is the common muscarinic link within the RSA generating system, a similar procedure should be also effective in the prevention of RSA induction by cholinergic stimulation of the hypothalamus (Gralewicz 1983) as well as by electrical stimulation of brain stem structures (Lindsley and Wilson 1975). The purpose of the experiment was to check the above supposition.

Methods

Nine adult male cats about two years old, weighing 2.5-3.5 kg, were used. The animals were prepared for the experiment by implanting them with intracerebral electrodes and cannulae.

SURGERY

The surgery was performed under barbiturate anaesthesia (hexobarbital, 80 mg/kg, i.p.). Stereotaxic coordinates were adopted from a stereotaxic atlas (Jasper and Ajmone-Marsan 1954). Each animal was implanted with two bipolar electrodes aimed at the dorsal hippocampus (Fr=4.5, L=5.0, H=+7.0), two bipolar electrodes aimed at the posteroventral hypothalamus (Fr= 9.0, L= 1.5, H=-3), or at periaqueductal gray substance (PAG): (Fr= 2.0, L=1.5, H=+1.0), and two monopolar, ball electrodes placed epidurally over frontoparietal cortical areas. The tips of the hippocampal recording electrodes were staggered vertically by 1.0-1.5 mm., and those of the hypothalamic or PAG stimulating electrodes by 0.5 mm. Apart from the electrodes, each of the animals was implanted with three guide cannulae, one aimed at the medial septum (Fr= 15.5, L= 0.5, H=+3) and two at the anterior hypothala-

mus, bilaterally (Fr= 12.5, L= 2.5, H=-2.0). The outlets of the cannulae were blocked with stainless steel stylets and sealed with a swab of cotton and sterile wax. The details of the electrode and cannulae manufacturing and of the surgery can be found in our previous paper (Gralewicz 1983). The animals were allowed at least 15 days for recovery.

APPARATUS

For the time of the experiment the animals were put into a cage (120 x 120 x 60 cm) with transparent top and front. EEG recordings were made with the use of an 8-channel electroencephalograph at 1-35 Hz frequency band. Electrical stimulation of the brain was performed with the use of a double square pulse stimulator and an isolation unit. Drugs were injected into the brain at 0.1 µl accuracy with the use of a microinjector (Zimmermann, GDR), joined directly with an injection cannula. The injection cannula was by 1.0 mm longer than the guide cannula.

DRUGS AND INJECTIONS

The following drugs were used: carbachol (carbamylcholine chloride, Fluka), atropine (atropine sulphate, Sigma), scopolamine (scopolamine hydrobromide, Sigma). The drugs were dissolved in a sterile, pyrogen-free physiological saline, so as to obtain the required concentration. The volume of the solutions injected into the brain was 1 µl in the case of CCh and 2.0 µl in the case of the cholinolytics. The duration of the injection (from the insertion to the withdrawal of the injection cannula) was about 1.0 min.

EXPERIMENTAL PROCEDURE

Each experimental session started with a 15 min control period during which EEG was recorded continuously. Then, an injection was made and the EEG recording was resumed immediately. In experimental sessions during which the effects of single injections were tested (CCh or a cholinolytic) the EEG was recorded for at least one hour after the

injection. In sessions with double injections (e.g. a cholinolytic followed by CCh) EEG was recorded for the whole period (i.e. 15 min) between the injections and then for at least one hour after the second injection.

In order to find out whether the blockade of muscarinic receptors within the medial septum does influence the spontaneous RSA generation as well as the induction of RSA by a cholinergic or electrical brain stimulation, the hippocampal activity was studied in the following conditions:

1. After intrahypothalamic injection of CCh (10 µg in 1.0 µl volume, unilaterally);
2. After intraseptal injection of At or Sc in a dose of 20 µg in 2 µl volume followed 15 min later by intrahypothalamic injection of CCh (10 µg, 1.0 µl);
3. After intraseptal injection of At or Sc (20 µg in 2.0 µl volume), repeated twice with a 15 min interval during the same session. Stimulation of posteroventral hypothalamus or PAG was done once every 10-15 min during the recording period;
4. After intraperitoneal injection of At (2.0 mg/kg) or Sc (0.2 mg/kg). Electrical brain stimulation was applied as above.

The doses of intrahypothalamic CCh (10 µg) and intraperitoneal At (2.0 mg/kg) were adopted on the basis of previous studies from our laboratory (Brudzyński 1981, Gralewicz 1981, Gralewicz 1983). The volumes and doses of intraseptal Sc were the same as applied by Bennett (1975). The brain loci for stimulation and the parameters of the stimulating current were established during one session performed several days before the start of the proper experiment. In this session the brain was stimulated once every five min with 5-10 s trains of biphasic rectangular pulses at 100 c/s frequency. The pulse duration was 0.2 ms. The peak to peak pulse amplitude was adjusted for each point separately. In a given animal the hypothalamic or PAG point yielding the best response (i.e. a continuous RSA in both hippocampi with signs of an increased behavioural arousal) was the one stimulated during the proper experiment. Except for rare occasions, the stimulation intensity once adjusted was kept constant during the successive testings.

The effects of each condition were tested once on each animal. The interval between successive testings was never shorter than seven days.

DATA ANALYSIS

Quantitative assessments were made only for records obtained from hippocampal derivations ipsilateral to the septal cannulae. The RSA content (i.e. the percentage duration of RSA) was calculated in 5 min EEG samples (the last 5 min before the injection and 10-15 min, 25-30 min, and 55-60 min after the injection) and in the fragments of records simultaneous to the electrical stimulation. In each case the amplitude of the largest three successive RSA waves, encountered in the analysed sample, was measured. The calculated mean was regarded as the maximum RSA amplitude. Less attention was

paid to the RSA frequency; changes in this parameter are strongly related to the animal behaviour, which could not be controlled precisely in this experiment.

At the end of the experiment the animals were sacrificed with an overdose of hexobarbital, the brains were removed from skulls, fixated in formalin and subjected to a standard histological procedure for verification of the electrode and cannulae placements.

Parametric and nonparametric analyses of variances were used for statistical evaluation of the obtained data (Siegel 1956, Winer 1962).

Results

HISTOLOGY

All injection sites in the septum were located between frontal planes 14.5 and 15.5 (Fig. 1). The tips of the recording electrodes were located within the dorsal hippocampus in all animals. The placements of hypothalamic and PAG stimulating electrodes did not differ more than 1 mm (in any direction) from the intended locations.

CHANGES IN HIPPOCAMPAL EEG AFTER INTRAHYPOTHALAMIC INJECTION OF CCH. EFFECT OF PRECEDING INTRASEPTAL INJECTIONS OF AT AND SC

The microinjections of Cch into the hypothalamus evoked in all cats a full set of symptoms of emotional-defensive response, which was described in details in previous papers from our laboratory (Brudzyński 1981, Gralewicz 1983). Changes of bioelectrical activity in the hippocampus consisted in an increase of RSA content (in some animals up to 100%). RSA frequency rose gradually, reaching the maximum 40-60 min after the injection. The dynamics of changes in both these parameters were studied in detail previously (Gralewicz 1983).

In no case At or Sc given intraseptally (20 μ g/2 μ l) prevented the RSA induction by the following injection of CCh into the hypothalamus, although the effect seemed to be slightly diminished in compari-

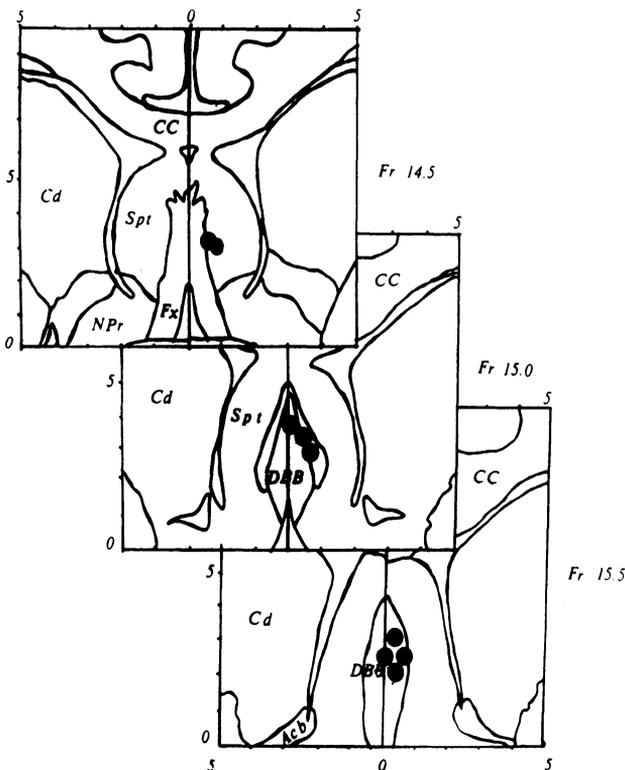


Fig. 1. Localization of injection sites (black circles) in the septum of the cat brain. Frontal sections (Fr) were taken from the Jasper and Ajmone-Marsan (1954) stereotaxic atlas.

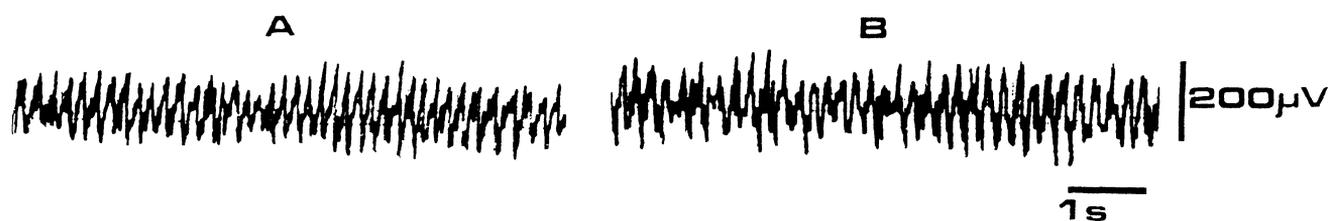


Fig. 2. Fragments of records of the hippocampal EEG made 15 min after intrahypothalamic injection of CCh (10 μ g) not preceded (A) and preceded (B) by intraseptal injection of At (20 μ g).

son with that observed after CCh alone (Fig 2. and Table I). A two-way ANOVA (Pretreatment type x Time) revealed that only the effect of Time was significant ($F_{1,13}=36.69$, $P<0.0001$); the RSA content before CCh injection was significantly lower than in all of the analysed 5-min sections of the hippocampal EEG after the injection ($P<0.05$ in all cases). The effect of pretreatment type as well as the interaction were not statistically significant.

The maximum RSA amplitude in different subjects was within the range of 300-750 μ V. The vari-

ability of this parameter in each subject was small and independent of the conditions of testing and the time after injections. Any statistical differences were not found.

Intraseptal injections of At or Sc, being ineffective in the prevention of the CCh induced RSA response, blocked completely the main symptom of the emotional-defensive response, i.e. growling. Autonomic signs, however, as well as signs of increased alertness were still evident after intrahypothalamic CCh.

TABLE I

Effects of intraseptal injections of At and Sc on changes in RSA content (in %) and growling induced by subsequent intrahypothalamic injections of CCh (10 μ g/1.0 μ l) in cats. (Values in parentheses are standard deviations)

Intraseptal injection preceding intrahypothalamic CCh injection		Hippocampal RSA and growing			
		Before intrahypothalamic CCh injection	10 - 15'	25 - 30'	55 - 60'
none (n=9)	RSA content	27.8 (6.0)	76.4 (14.3)	85.7 (8.7)	80.0 (10.5)
	Growling	-	+	+	-
Atropine 20 mg/2 ml (n=3)	RSA content	16.0 (7.4)	35.8 (11.5)	54.3 (13.7)	66.7 (18.3)
	Growling	-	-	-	-
Scopolamine 20 mg/2 ml (n=6)	RSA content	24.8 (7.6)	54.8 (13.5)	61.8 (10.8)	66.2 (15.7)
	Growling	-	-	-	-

EFFECTS OF INTRASEPTAL AND INTRA-PERITONEAL INJECTIONS OF ATROPINE OR SCOPOLAMINE ON SPONTANEOUS OR ELECTRICALLY INDUCED HIPPOCAMPAL RSA

Data from this part of the experiment are presented in Table II. Good RSA response, meeting the established criteria, was obtained in six cats. In no case the first intraseptal injection of At (20 µg/2µl) or Sc (20µg/2µl) resulted in clear-cut changes in the spontaneous as well as in the electrically induced RSA during the 15 min between the first and the second injection. Ten to 15 min after the second injection the content of the spontaneous RSA started to decline in some cats and a characteristic "sleep-like" pattern of activity appeared in the hippocampal as well as in the cortical EEG. The differences in RSA content between successive recording periods, however, attained statistical significance in the case of Sc only.

Contrary to the spontaneous RSA, the RSA produced by electrical brain stimulation showed no changes after the first nor after the second injection of cholinolytics into the septum (Table II A and B,

Fig 3B). 2.0 mg/kg At or 0.2 mg/kg Sc, given intraperitoneally, eliminated spontaneous RSA as soon as 10-15 min after the injection and rendered electrical brain stimulation (PAG and posterior hypothalamus) ineffective (Table II C); RSA during stimulation did not appear even when the stimulation intensity was doubled (Fig. 3C).

Discussion

The above results have shown that At or Sc, when administered directly into the medial septum, do not prevent RSA induction by CCh stimulation of the hypothalamus as well as by electrical stimulation of PAG or posterior hypothalamus. They do prevent, however, the most prominent effect of intrahypothalamic CCh, i.e. the growling. The lack of effect of intraseptal At or Sc on RSA is in contrast with the relatively fast and efficient elimination of this rhythm, either spontaneous or provoked, when these drugs were administered intraperitoneally. It also does not support Bennett's (1975) observations in spite of the fact that the doses and volumes used in our experiments were similar to those in Bennett's studies. It should be noted, however, that

TABLE II

Changes in the mean percentage content of spontaneous and electrically induced RSA after intraseptal or intraperitoneal injections of cholinolytics. *a*, spontaneous RSA. Values refer to the whole 5 min. sample. *b*, RSA induced by electrical brain stimulation. Values refer to the EEG sections parallel to the stimulation

Cholinolytic: dose and administration route			RSA content in the hippocampal EEG after injection				Results of Fridman ANOVA
			Control	10-15'	25-30'	55-60'	
A	Atropine intraseptally 20 µg/2 µl x 2 (n=6)	a	33.0	35.0	27.2	21.0	X=6.3 NS
		b	86.6	86.1	88.1	86.1	X=0 NS
B	Scopolamine intraseptally 20 µg/2 µl x 2 (n=6)	a	31.2	16.6	12.8	1.6	X=10.5 p<0.02
		b	80.8	76.2	70.8	74.6	X=2.2 NS
C	Atropine i. p. 2.0 mg/kg or scopolamine 0.2 mg/kg (n=6)	a	38.0	0	0	0	Not analysed
		b	91.0	2.5	0	0	Not analysed

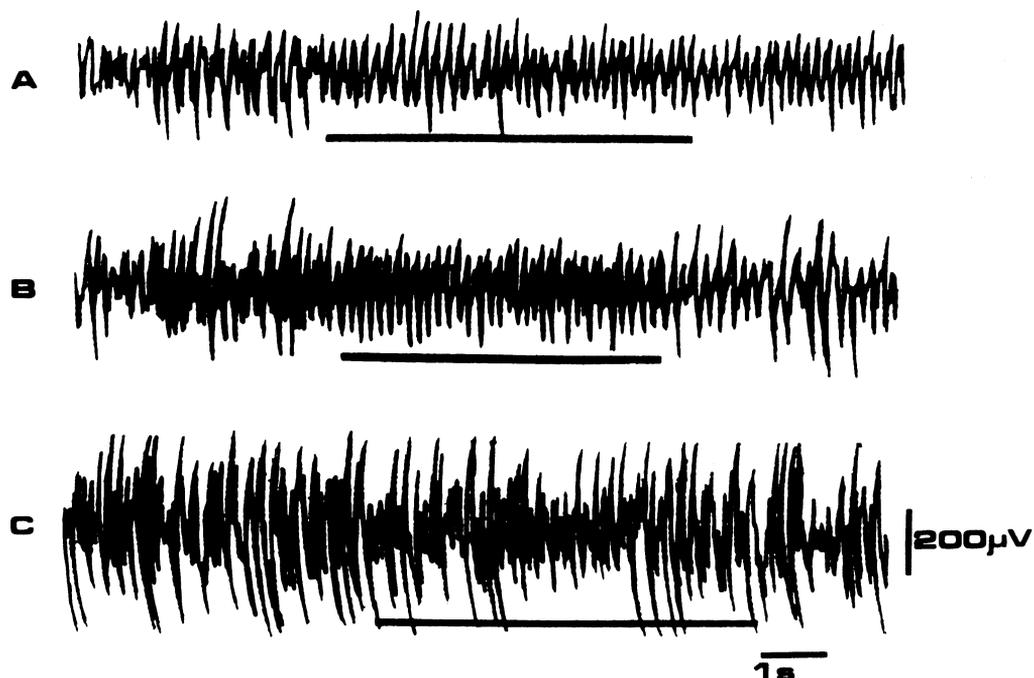


Fig. 3. Fragments of records of the hippocampal EEG illustrating the effects of intraseptal and intraperitoneal injections of cholinolytics on RSA induction by electrical stimulation of the posterior hypothalamus ($150 \mu\text{A}$, $100/\text{s}$, 0.2 ms). *A*, before intraseptal injection of scopolamine. *B*, about 15 min. after the second injection of scopolamine ($20 \mu\text{g}$). *C*, about 20 min. after intraperitoneal injection of atropine (2.0 mg/kg). The continuous lines under the records mark the stimulation period.

RSA in Bennett's experiments was not totally abolished; after intraseptal Sc about 6% of instrumental responses (approaching a feeder) were still accompanied by this form of activity.

Within the brain, the drugs can diffuse and reach loci quite distant from the place of injection (Myers et al. 1971). Nonetheless, a possibility should be considered that in the present experiments the effective concentration of At or Sc within the septum might have been limited to an area too small to encompass all cholinceptive (muscarinic) elements, which take part in RSA generation. To make the situation clear, the second experiment was performed.

EXPERIMENT II

Intraseptal injections of CCh in cats may produce an emotional-defensive response which resembles that after intrahypothalamic injections (Brophy and Levitt 1974). It is also accompanied by a continuous RSA, providing the dose of CCh was

not high enough to provoke epileptic seizures (McLean 1957 and personal observations). The purpose of the second experiment was to find out whether the RSA induced by intraseptal injection of CCh could be prevented or blocked by an injection of At or At and hexamethonium (Hx), a nicotinic blocker, into the same locus.

METHODS

Three adult male cats were used. Bipolar electrodes were implanted in the dorsal hippocampus and cannulae into the medial septum according to the same stereotaxic coordinates as in the first experiment. Starting two weeks after the surgery, the hippocampal EEG was studied in the following conditions: (1) before and after intraseptal injections of $2.5 \mu\text{g}$ CCh (2) before and after an intraseptal injection of At ($5.0 \mu\text{g}$) followed 15 min later by an injection of $2.5 \mu\text{g}$ CCh into the same locus; (3) before and after an intraseptal injection of $2.5 \mu\text{g}$ CCh followed 15 min later by an injection of

TABLE III

Effect of intraseptal injection of At on changes in RSA content (in %) and growling induced by subsequent intraseptal injection of CCh (2.5 µg/0.5 µl) in cats ($n=3$). Values in parentheses are standard deviations

Intraseptal injection preceding intraseptal injection of CCh		Hippocampal RSA and growling				
		Control	10-15 ' after preceding injection	After intraseptal CCh injection		
				10-15'	25-30'	55-60'
None	RSA content	41.2 (7.8)		73.4 (10.2)	83.1 (6.2)	89.3 (4.6)
	Growling	-		+	+	-
Atropine 5 µg/0.5 µl	RSA content	38.6 (6.4)	35.7 (7.7)	58.1 (8.4)	74.4 (7.4)	78.0 (6.2)
	Growling	-	-	-	-	-

10 µg At and then after another 10 min an injection of 10.0 µg Hx into the same locus. (Additional injections after CCh injection appeared possible if the cat was approached very slowly and treated gently). Under the latter conditions the cats were tested twice. The doses for CCh and At were chosen on the basis of some pilot studies; 2.5 µg CCh sufficed for induction of the emotional-defensive response and 5.0 µg At was sufficient to prevent it. The ratios between CCh, At and Hx doses were similar to those used in the case of hypothalamus, where effective prevention of RSA was observed in the majority of cats when the At and Hx doses exceeded that of CCh by at least three times each (Gralewicz 1983). The volume of injected solutions was 0.5 µl in each case.

Results

The locations of the cannulae within the septal region of the three cats used in this experiment were similar as shown in Fig 1.

EFFECTS OF CCH INJECTIONS

In all cats intraseptal injections of CCh resulted in changes in hippocampal EEG and behaviour.

Changes in the EEG consisted in a marked increase of RSA content. Changes in behaviour consisted in the appearance of emotional-defensive response, similar to that after intrahypothalamic CCh injections. Sixty min after the injection the RSA content showed no tendency to decline. The emotional defensive response (growling) subsided within 30-45 min.

EFFECTS OF AT AND HX ON THE EFFECTIVENESS OF CCH IN THE SEPTUM

No changes in behaviour and hippocampal EEG were observed up to 15 min after the intraseptal injection of 5 µg of At. CCh injected into the same locus 15 min after At resulted in an increase of RSA content almost to the same level as in the session with no preceding At injection and remained so until the end of recording (60 min). The emotional defensive response, however, did not appear (Table III). At (10 µg) injected intraseptally 15 min after intraseptal CCh had no effect on RSA, but the growling disappeared almost immediately. Hx injected additionally 10 min after the At injection resulted in no overt change, i.e. the RSA remained increased till the end of the recording (Table IV).

TABLE IV

Effect of CCh (2.5 µg), At (10 µg) and Hx (10 µg) injected sequentially into the septum on RSA content and growling. The sequence was repeated twice in each of the three cats. Values in parentheses are standard deviations

	Control	10-15' after CCh	10-15' after At (20-25' after CCh)	5-10' after Hx (30-35' after CCh)	25-30' after Hx (50-55' after CCh)
RSA content	36.1 (6.1)	74.2 (9.2)	74.4 (9.2)	70.7 (11.3)	68.1 (10.8)
Growling	-	+	-	-	-

Discussion

The results of the second experiment show that whereas intraseptal At prevents or blocks the behavioural effect of intraseptal CCh, i.e. growling, it is not effective in blocking the concomitant electroencephalographic response, i.e. hippocampal RSA. What is more, an additional intraseptal injection of Hx after the injection of At does not change the result. It suggests that CCh, given intraseptally, influences hippocampal EEG through the activation of cholinergic receptors belonging to several classes, some of which are blocked neither by At nor by Hx.

GENERAL DISCUSSION

The results obtained in the second experiment resemble to some degree our earlier data concerning the hypothalamus (Gralewicz 1983). In both cases intraseptal At preceding intraseptal CCh blocked effectively the defensive-emotional response, but not the RSA response. In the case of hypothalamus the RSA response could be prevented by a mixture of At and Hx preceding CCh, but not in all animals; in some a continuous RSA still appeared in spite of heavy doses of At and Hx. Thus, these data suggest that in the cat CCh given intrahypothalamically, as well as intraseptally, induces RSA through the activation of cholinergic receptors belonging to several classes.

The range of frequency of the cat RSA as well as its sensitivity to At given systemically suggested its identity with the type 2 RSA of rodents (Bennett

et al. 1978). In rats, however, the type 2 RSA may be blocked not only by systemic but also by direct intraseptal application of At (Stewart and Fox 1989). Thus, the persistence of RSA after intraseptal application of At in the cat suggests that the neurochemistry of the septal part of the RSA generating mechanism in the cat differs from that responsible for type 2 RSA in the rat.

Summing up, the results obtained in both experiments described above suggest that medial septum is not a common muscarinic link within the RSA generating system of the cat. The fact remains, however, that systemic injection of At or Sc can completely block RSA in this species (Tori and Wikler 1966, Gralewicz 1981). It is possible that the suggested common muscarinic link is located in the hippocampus itself. A direct application of CCh to the rat hippocampus *in vivo* (Rowntree and Bland 1986), as well as, *in vitro* (Konopacki et al. 1987), may induce a RSA-like activity and this effect is completely antagonized by subsequent infusion of At. Years ago McLean (1957) showed that direct cholinergic stimulation of the cat hippocampus may result in a continuous RSA. It is not known, however, whether this response, similarly as that in the rat, can be prevented by intrahippocampal injection of muscarinic cholinolytics. Another possibility is that there is no common muscarinic link (in a sense of group of neurones located in a defined area) in the RSA generating system of the cat. As our previous (Gralewicz 1983) and present data suggest, several classes of cholinergic receptors participate in the induction of RSA response by CCh injected intrahypothalamically or intraseptally. It is conceivable

that the transmission of impulses along the multisynaptic pathways from MRF to the hippocampus is mediated in each relay station not through the same class of cholinergic receptors. The cholinergic neurones with muscarinic receptors might be randomly distributed within this pathway, but in such a way that each single transmission line had at least one muscarinic relay station. Such organization could explain the complete elimination of RSA by At or Sc when administered systemically, but not when injected into defined loci along the reticulosepto-hippocampal pathway.

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