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Intraventricular Anti-cholinergics Do Not Block Cholinergic Hippocampal RSA or Neocortical Desynchronization in the Rabbit or Rat[1]

IAN Q. WHISHAW, TERRY E. ROBINSON AND TIMOTHY SCHALLERT

Department of Psychology, University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4

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WHISHAW, I. Q., T. E. ROBINSON AND T. SCHALLERT. *Intraventricular anti-cholinergics do not block cholinergic hippocampal RSA or neocortical desynchronization in the rabbit or rat.* PHARMAC. BIOCHEM. BEHAV. 5(3) 275–283, 1976. – Electroencephalographic (EEG) electrodes and ventricular cannulae were implanted in 8 rabbits and 12 rats. Two anti-cholinergic agents, atropine sulfate and scopolamine hydrobromide, were given systemically (1–50 mg/kg) and intraventricularly (5–800 μg). Systemic but not intraventricular injections blocked sensory stimulation-induced or eserine-induced neocortical desynchronization and hippocampal RSA in rats and rabbits which were immobile and either undrugged or ethanol intoxicated. Systemic injections also blocked hippocampal RSA but not neocortical desynchronization in rats given sensory stimulation under urethane anaesthesia, while intraventricular injections only reduced RSA amplitude. Neither systemic nor intraventricular injections blocked neocortical desynchronization or hippocampal RSA recorded from animals when they walked in a motor driven wheel. These experiments support the hypothesis that there are two types of neocortical desynchronization and hippocampal RSA, one cholinergic and one non-cholinergic. They also suggest that atropine and scopolamine pass more readily to the neural system responsible for cholinergic EEG activity from the capillary bed than from the ventricular fluid.

Anti-cholinergic-sensitive EEG Eserine Urethane Anti-cholinergic resistant EEG Hippocampal EEG
Ventricular injection Atropine Neocortical EEG Ethyl alcohol Scopolamine

HIPPOCAMPAL rhythmical slow activity (RSA or theta rhythm) is produced by two anatomically separate generators in the hippocampal formation, one located proximal to the pyramidal cells in regio superior of CA-1 and the other proximal to the granule cells in the dentate gyrus [3, 5, 34]. Since RSA in the two generators is 180° out of phase and a null zone separates them, bipolar electrodes with a tip located in each generator will record the summed activity yielding RSA with amplitude as large as 3 mV [5].

A number of studies using RSA frequency analysis, behavioral observation, and pharmacological manipulation suggest that at least two types of RSA [11, 18, 29] can be obtained from either the CA-1 or dentate gyrus generators [31]. In the rat one type of RSA is characterized primarily by its occurrence during behavioral immobility (especially in response to sensory stimulation), its activation by eserine, and its usual frequency of 4–7 Hz. Such RSA is abolished by anti-cholinergic agents but is resistant to diethyl ether, urethane, or alcohol anaesthesia. The other type of RSA is characterized primarily by its occurrence during the performance of voluntary [27,33] or Type I [28] movements such as walking, swimming, or rearing and its normal frequency of 6–12 Hz. This latter type of RSA is blocked by diethyl ether, urethane, or alcohol anaesthesia but is resistant to large doses of anti-cholinergic agents. The two types of RSA can be termed slow vs fast, immobility vs mobility-related, and anti-cholinergic sensitive vs anti-cholinergic resistant. The first two terms can be misleading since under conditions such as hypothermia [32] or muscular paralysis [31], fast RSA can be slowed or elicited during immobility. For this reason the two types of RSA are best defined as anti-cholinergic sensitive and anti-cholinergic resistant.

Paralleling the two types of RSA are two types of neocortical desynchronization, or low voltage fast activity [11, 18, 28, 29]. One type occurs during behavioral immobility, is sensitive to anti-cholinergic agents, and is resistant to urethane, ether, and ethanol. The other type occurs during voluntary movement and it is resistant to anti-cholinergic agents but sensitive to ether and ethanol.

It has been postulated that the blocking action of atropine on EEG is central since atropine methyl nitrate, which has an equivalent peripheral action but does not cross the blood-brain barrier, does not block EEG activity or either type. However, large doses of atropine must be administered by intraperitoneal or intravenous routes to produce EEG changes. Smaller doses are required to produce peripheral changes such as mydriasis. This suggests

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that the permeability of the brain to atropine sulfate is not great, a conclusion supported by other evidence [1, 14, 17, 26]. For example, Tønnesen [26] found that after an intravenous injection (5 mg/kg) of atropine sulfate in rats, brain tissue contained less than 2 μg/g while the lungs contained 60 μg/g and the heart 12 μg/g. One way of bypassing the blood-brain barrier while avoiding peripheral action might be to infuse atropine directly into the ventricles. Substantial evidence indicates that the permeability of brain tissue is greater to substances injected by intraventricular as compared to intravenous routes [2, 8, 12, 13]. On the basis of brain tissue content analysis [26], EEG findings [18, 28, 30], and the brain weight of rat and rabbit it could be argued that if all atropine were evenly distributed to brain tissue from the ventricles, then an infusion of 6 μg of atropine into the rat and 20 μg of atropine into the rabbit would be sufficient to block anti-cholinergic sensitive EEG activity.

The purpose of this study was to test the hypothesis that atropine has a central action by infusing atropine into the ventricles of the rat and rabbit. The results show that anti-cholinergic sensitive EEG was not blocked, even by very high doses, which suggests that if the site of action is central it is inaccessible to atropine given via this route. The results also show that EEG recording can be used for comparing the effectiveness of drugs given via different routes.

METHOD

Animals

The experiments were performed with 8 adult, male New Zealand rabbits and 12 adult, male Sprague Dawley rats.

Surgical Procedure

Animals were anaesthetized with sodium pentobarbital and implanted with intracranial electrodes and ventricular cannulae using standard stereotaxic techniques. Each animal was implanted with a bipolar electrode in the frontal neocortex and a bipolar or tripolar electrode bilaterally in the dorsal hippocampus. Ventricular cannulae were implanted bilaterally in the rabbits; one cannula was positioned in the dorsal arm of one lateral ventricle and one in the ventral arm of the contralateral ventricle. A silastic tube with an end attached to the head assembly was also implanted in the external jugular vein. In rats, a single cannula was implanted in a lateral ventricle. Cannulae and electrodes were fixed in place with dental cement anchored to jeweler's screws inserted in the skull. A period of 1–3 weeks was allowed for recovery from surgery.

Recording Electrodes

Recording electrodes consisted of three 254 μ dia. Nichrome wires insulated except for the cross section of the tips, which were staggered vertically by 1.0–1.5 mm. The pair of wires which later yielded the largest amplitude and clearest RSA was used to take hippocampal records during subsequent experiments. The bipolar electrodes placed in the neocortex had wires with one tip cut 1 mm shorter than the other to permit surface to depth recording [28]. Coordinates for hippocampal electrodes in rabbits were: posterior to bregma 4.5 mm, lateral 5.5 mm, and ventral to the horizontal surface of the skull 6.5 mm. Coordinates for hippocampal electrodes in rats were: 4.0, 2.5, and 3.3–3.5 mm. Winchester male connectors were attached to the wires for contacts. A male connector attached to a jeweler's screw inserted in the frontal bone served as a ground.

Ventricular Cannulae

Each intraventricular cannula consisted of a guide tube, stylet, and injection tube. The guide tube was constructed of 21 ga. stainless steel tubing, the tip of which was cut to a 1 mm bevel. When not in use the guide tube was blocked with a stylet made from a No. 3 Ward's insect pin cut to length. The injection cannula was a 25 ga. stainless steel tube which penetrated the guide tube to the level of the bevel. The fit of the injection tube was made sufficiently snug to prevent seepage. The cannula was implanted with the injection tube in place and connected to a 10 cm length of PE-20 tubing filled with artificial cerebrospinal fluid (CSF). The cannula was lowered with a microdrive unit and tip placement in the ventricle was verified when the CSF in the upraised tube began to flow (for a description of the technique see Myers, [21]).

The animals were connected to a Grass Model 7C polygraph with Beldon 8429-250 shielded phono-arm cable. Connections to the head assembly were made with Winchester subminiature female connectors. EEG activity was recorded with half-amplitude filters set at 1 and 75 Hz. Each hippocampal record was displayed on a second channel after being passed through a Dytronics filter with band pass settings at 2 and 12 Hz. A manually activated marker was used to indicate behavior on the chart. Test sessions lasted from 2 to 3 hr for each animal and EEG activity was recorded continuously in each session. Records were analyzed using a clear plastic ruler (mm scale) which measured frequency and amplitude of individual waves from samples of at least 1 min duration as described by Whishaw and Vanderwolf [33].

Drugs

Ethyl alcohol was mixed as a 50% solution in sterile distilled water and infused intravenously into rabbits in 2 mg/kg doses at a rate of 1 ml/min with a constant rate pump. Atropine sulfate was given intravenously to rabbits as a 5 mg/kg dose. The following drugs and dosages were given intraperitoneally to rats: physostigmine salicylate (eserine), 0.5–1.0 mg/kg; ethyl carbamate (urethane), 1 g/kg; chlorpromazine hydrochloride, 2.5 mg/kg; atropine sulfate, 50 mg/kg; atropine methyl nitrate, 10 mg/kg. For central injection atropine sulfate (given to rabbits and rats) and scopolamine hydrobromide (given to rats only) were dissolved in artificial CSF (see Myers [20], p. 65). Atropine was mixed as a 10 μg/μl solution (pH 7.1) and scopolamine as a 1 μg/μl (pH 7.5) solution and each was infused at a rate of 5 μl/min with a constant rate pump. Four successive doses of 50 μg of atropine and four successive doses of 5 μg of scopolamine were given in the major experiments. Higher doses of atropine (up to 800 μg in 200 μg/μl concentration; pH 7.4) were also given in a single treatment to a few animals.

Procedure

Rabbits. Neocortical and hippocampal EEG were recorded from 8 rabbits during 3 behavioral conditions: (a) hopping at 10 m/min in a motor driven wheel of the type

described by Whishaw and Vanderwolf [33], (b) sitting motionless and undisturbed, and (c) being stroked or turned when otherwise motionless. The procedure of turning the animals was used when they were intoxicated with ethanol [30].

A sequence of four ventricular injections (50 μg) of atropine sulfate was administered to 6 rabbits twice under 2 different conditions, (a) when they were otherwise normal, and (b) when they were intoxicated with an intravenous injection of 2 mg/kg ethanol. The order of the normal and ethanol conditions was counterbalanced and the atropine injection sequences were alternated between the two ventricular cannulae. Each 50 μg dose of atropine was given at 10 min intervals. Thirty minutes after the last ventricular injection, each rabbit received a 5 mg/kg intravenous injection of atropine sulfate. Before the first injection and after each injection, the EEG and behavioral observations were recorded. A period of 7–14 days was allowed for recovery between each condition in which a ventricular atropine sequence was given.

Two additional rabbits received the same behavioral tests under normal and ethanol intoxication conditions, and then received a 400 μg injection of atropine sulfate in each cannula.

Rats. Neocortical and hippocampal EEG were recorded from rats during 3 behavioral conditions: (a) walking in the motor driven wheel at a speed of 10 m/min, (b) standing or lying motionless, and (c) when pinched lightly on the base of the tail while anaesthetized with urethane.

A sequence of four intraventricular injections of atropine sulfate (50 μg) was given to 6 rats under 3 different conditions: (a) when they were otherwise normal, (b) 10 min after they were pretreated with 0.5 or 1.0 mg/kg of eserine (In order to reduce the vigor of tremors and salivation produced by eserine, 2.5 mg/kg chlorpromazine and 10 mg/kg atropine methyl nitrate were given intraperitoneally 20 min prior to the administration of eserine [28]), and (c) when they were anaesthetized with 1 g/kg of urethane. The order of the normal, eserine, and urethane treatments was counterbalanced and a period of 7–14 days was allowed for recovery between each condition in which a ventricular sequence was given. Each 50 μ dose of atropine was given at 10 min intervals. Thirty minutes after the last ventricular injection each rat received a 50 mg/kg intraperitoneal injection of atropine. Before the first injection and after each injection, the EEG and behavioral observations were recorded.

Three additional groups of 2 rats each were given a sequence of four intraventricular injections of scopolamine (5 μg) under one of three different conditions: (a) when they were otherwise normal, (b) following pretreatment with eserine as described above, or (c) following urethane anaesthetization as described above. Thirty minutes after the last central injection an intraperitoneal 5 mg/kg injection of scopolamine was given.

Following 8 days of recovery, 2 of the latter 6 rats were given eserine (0.5 and 1.0 mg/kg) followed by a single intraventricular dose of 500 μg atropine sulfate.

Histology

At the completion of the experiments the animals were deeply anaesthetized with sodium pentobarbital and a 5 μl volume of saturated Eosin red dye was injected into each ventricle (rabbits received 5 μl of thionin in the second cannula). The animals were then perfused with saline followed by a 10% Formalin solution. The brains were removed and stored in 10% Formalin for 24 hr before being frozen, cut in 40 μ sections, mounted and stained with cresyl violet or thionin for histological examination.

RESULTS

Histological analysis indicated that the cannulae were successfully implanted with the tips in the lateral ventricles. Figure 1 shows a cannula placement in the lateral ventricle of a rabbit. Other evidence of successful localization included the following observations: (a) when stereotaxically placed, there was a pressure-induced flow of artificial CSF, (b) when the stylet was removed for use, CSF flowed from the cannulae, and (c) injected dye was found distributed throughout the ventricular cavities. Histological analysis also showed that the recording electrodes were placed with tips in the hippocampal formation confirming that large amplitude RSA (up to 2.5 mV in these experiments) is produced by generators in this structure [5].

A summary of experimental treatments and the results of ventricular atropine and scopolamine injection are shown in Table 1. Intraventricular injections abolished neither the movement related nor the immobility related neocortical desynchronization and hippocampal RSA. Intravenous and intraperitoneal injection of the anti-cholinergic agents had little effect on movement related neocortical desynchronization and hippocampal RSA but in all experimental conditions these treatments blocked neocortical desynchronization and hippocampal RSA which occurred during immobility.

Effects of Atropine and Alcohol on Rabbit EEG

Hippocampus. The mean frequency and standard deviation of RSA obtained when rabbits hopped in the wheel was 7.2 ± 0.5 Hz; mean amplitude was 1.4 ± 0.4 mV. Frequency was unchanged after intraventricular and intravenous atropine. Intraventricular atropine produced a slight decline in the amplitude of movement related RSA (range of amplitude reduction 12–36%) in the hippocampus ipsilateral to the injection cannula in 6/12 tests and a 16% bilateral amplitude reduction in 1/12 tests. Intravenous injections also produced a slight amplitude reduction in both hippocampal sites (mean control = 1.4 mV, range = 0.9–2.5 mV, vs mean atropine = 1.2 mV, range = 0.7–2.3 mV). When the animals were still and receiving sensory stimulation, the mean frequency (with standard deviation) of RSA was 6.4 ± 2 Hz, and the mean amplitude was 1.1 ± 0.5 mV. When the animals were immobile and being stroked or turned during ethanol intoxication, the mean frequency of RSA was 5.3 ± 0.3 Hz, and the mean amplitude was 1.1 ± 0.5 mV. Intraventricular atropine produced no change in frequency of immobility related RSA, but reduced amplitude in the hippocampus ipsilateral to the injection cannula in 6/12 tests with undrugged rabbits, and in 5/12 tests with intoxicated rabbits. In each of the behavioral conditions mean amplitude reduction was less than 20%. Intravenous atropine abolished all immobility related RSA with a latency of 2–3 min.

Neocortex. Intraventricular atropine produced no obvious change in the desynchronized neocortical EEG. After atropine was given intravenously this desynchronized pattern was present when the animals moved, but was

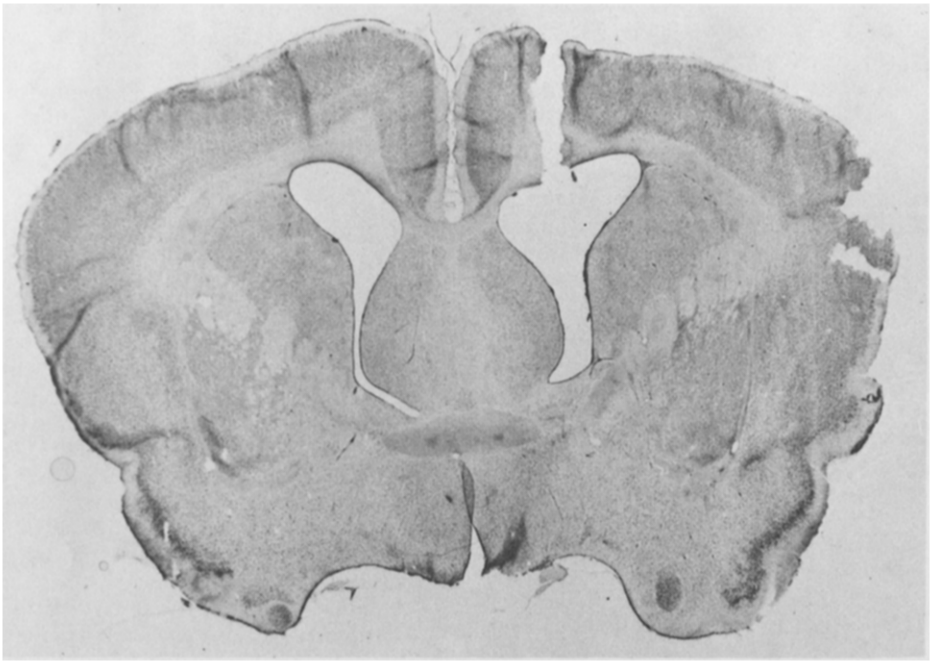

FIG. 1. Coronal section of a rabbit brain cut at 40 μ showing cannula tract to the ventricle. Cresyl violet stain.

TABLE 1

EFFECTS OF ANTI-CHOLINERGIC AGENTS (ATROPINE, SCOPOLAMINE) ON INCIDENCE OF RHYTHMICAL SLOW ACTIVITY (RSA) DURING MOVEMENT AND DURING IMMOBILITY WITH SENSORY STIMULATION UNDER NO-DRUG, ESERINE, URETHANE AND ALCOHOL CONDITIONS IN THE RAT AND RABBIT

		Number of Animals with RSA Present During Movement	Number of Animals with RSA Present During Sensory Stimulation and Immobility
No Anti-cholinergic			
Rat			
	No drug	8/8	No observation
	Eserine	8/8	8/8
	Urethane	No movement	6/6
Rabbit			
	Normal	6/6	6/6
	Ethanol	No movement	6/6
Ventricular Anti-cholinergic			
Rat			
	No drug	8/8	No observation
	Eserine	10/10	10/10
	Urethane	No movement	6/6
Rabbit			
	Normal	12/12	12/12
	Ethanol	No movement	14/14
Intraperitoneal Anti-cholinergic			
Rat			
	No drug	8/8	No obeservation
	Eserine	10/10	0/10
	Urethane	No movement	0/6
Rabbit			
	Normal	12/12	0/12
	Ethanol	No movement	0/14

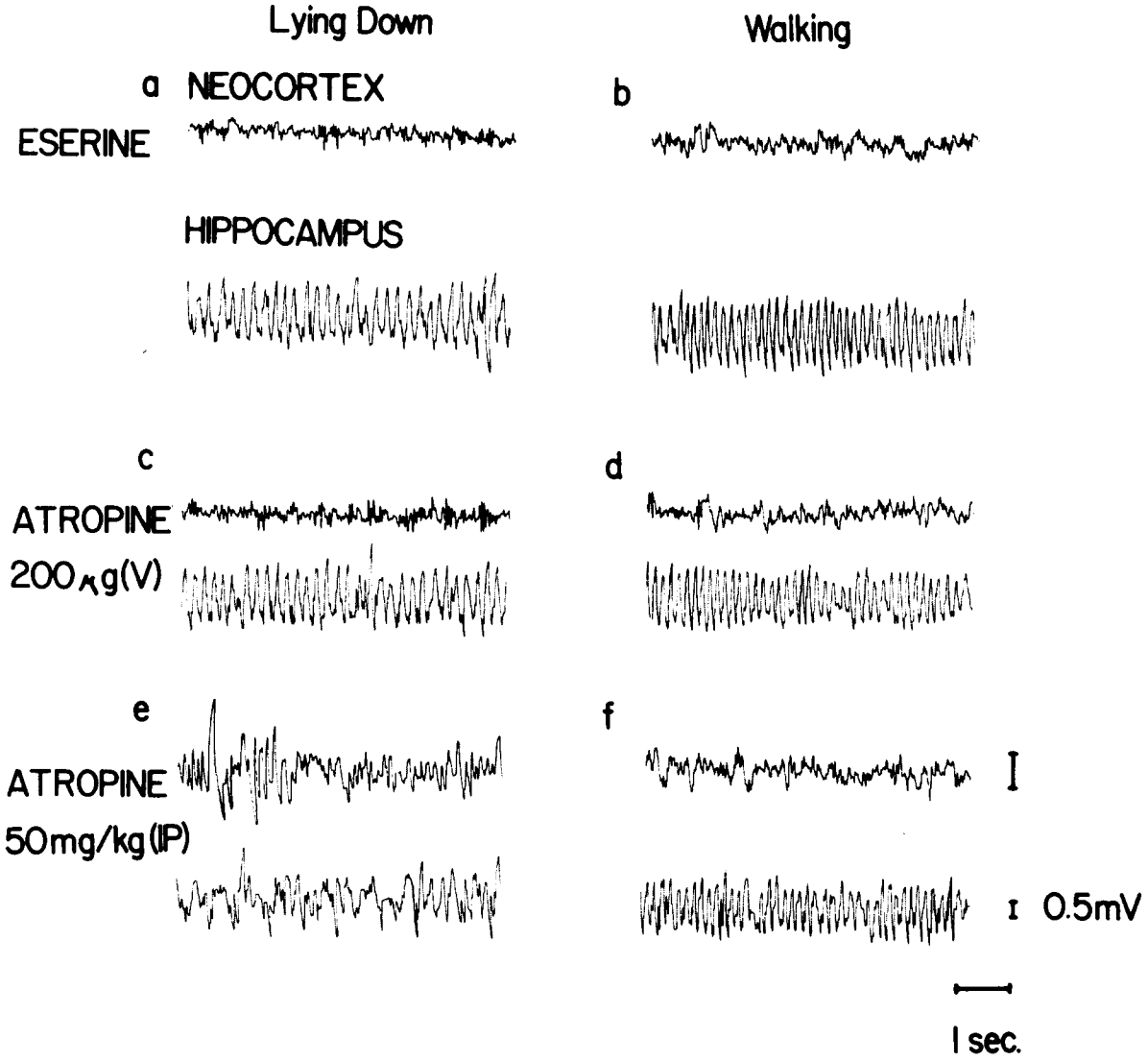

FIG. 2. Effects of intraventricular and intraperitoneal atropine sulfate on neocortical and hippocampal EEG in the eserinized rat during lying down (left) and walking in the motor driven wheel (right). Note: (1). In a, desynchronized neocortical EEG and slow hippocampal RSA (5 Hz) during lying down and, in b, desynchronized neocortex and faster (7 Hz) RSA during walking in the wheel. (2). In c and d, 20 min after 200 μg of atropine sulfate given intraventricularly, EEG during both behaviors is essentially unchanged. (3). In e, 15 min after a 50 mg/kg intraperitoneal injection of atropine sulfate, the desynchronized neocortical pattern and slow hippocampal RSA associated with lying down is abolished, but in f, the desynchronized neocortex pattern and fast hippocampal RSA is still recorded during walking in the wheel.

absent when they were immobile and given sensory stimulation.

Effects of Eserine and Anti-cholinergic Agents on Rat EEG

An example of EEG obtained from an eserinized rat which received atropine first via an intraventricular route and then via an intraperitoneal route is shown in Fig. 2. When an eserinized animal was lying down the neocortex was desynchronized and RSA with mean frequency of 5.3 ± 0.4 Hz was recorded. The animals were never completely still, however, since eserine produced marked and extensive tremors and twitches. When the wheel was turned and the animals walked, RSA frequency rose to 7.3 ± 0.4 Hz. These results are depicted in Fig. 2a and b. Intraventricular injections of atropine or scopolamine produced no significant changes in neocortical or hippocampal EEG activity (Fig. 2c and d). Intraperitoneal injections of atropine sulfate (50 mg/kg) abolished the neocortical desynchronization and hippocampal RSA when the animals were lying down (Fig. 1e). However, the neocortical desynchronization and hippocampal RSA were clearly present when the animals were walking in the wheel (Fig. 1f).

Effects of Urethane and Anti-cholinergic Agents on Rat EEG

Examples of EEG obtained from a rat anaesthetized with urethane is shown in Fig. 3. When the animals were stimulated with a tail pinch, neocortical EEG desynchronization and RSA with a mean frequency of 5.2 ± 0.3 Hz were recorded. After 100 μg of intraventricular atropine,

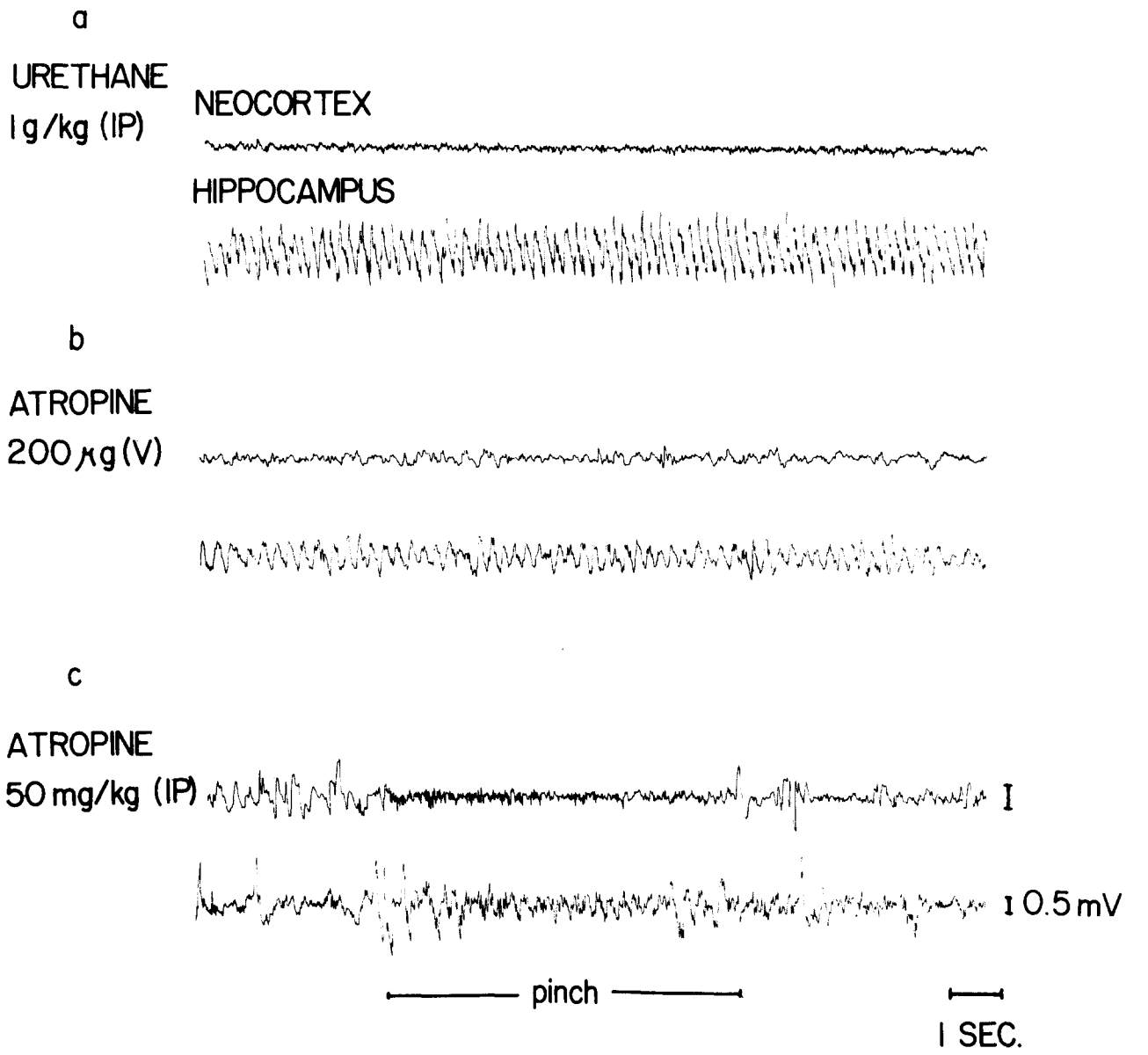

FIG. 3. Effects of intraventricular and intraperitoneal atropine sulfate on neocortical and hippocampal EEG in a urethane treated rat. Note: In a, the desynchronized neocortical record and slow (5 Hz) hippocampal RSA during urethane anaesthesia. In b, after 200 μg intraventricular atropine sulfate, neocortical EEG is still desynchronized but the amplitude of hippocampal RSA is greatly reduced. In c, 20 min after 50 mg/kg intraperitoneal atropine sulfate, both neocortical and hippocampal patterns are irregular. In response to sensory stimulation the neocortical record shows a desynchronized pattern but the hippocampal record no longer shows RSA.

the amplitude of RSA fell from 1.1 ± 0.5 mV to 0.4 ± 0.12 mV. This decrease was highly significant ($p<0.001$). The amplitude of RSA showed no further reduction as intraventricular doses were raised to 200 μg (Fig. 3). Neocortical EEG remained desynchronized during tail pinches after intraventricular atropine or scopolamine.

Figure 3c shows the effect of intraperitoneal atropine on EEG of the urethanized rat. Large amplitude slow waves were present in neocortical and hippocampal records when the animals were undisturbed. In response to sensory stimulation (tail pinch) neocortical EEG was still desynchronized but RSA was no longer present in the hippocampal record. The results obtained after scopolamine were similar.

Effects of Large Intraventricular Doses of Anti-cholinergic Agents

Two ethanol intoxicated rabbits received an intraventricular 800 μg (400 μg in each cannula) injection of atropine and two eserinized rats received single 500 μg doses. In each case the results were similar. Neocortical desynchronization and hippocampal RSA were still present, and large amplitude (up to 5 mV) spike-shaped waves occurred in the hippocampal record. The spikes occurred at intervals of 2–10 sec and continued for periods lasting up to 2 hr. An example of a record obtained from a rat is shown in Fig. 4. In preliminary experiments, still higher doses of central atropine were tried but in each case full behavioral electrographic seizures occurred.

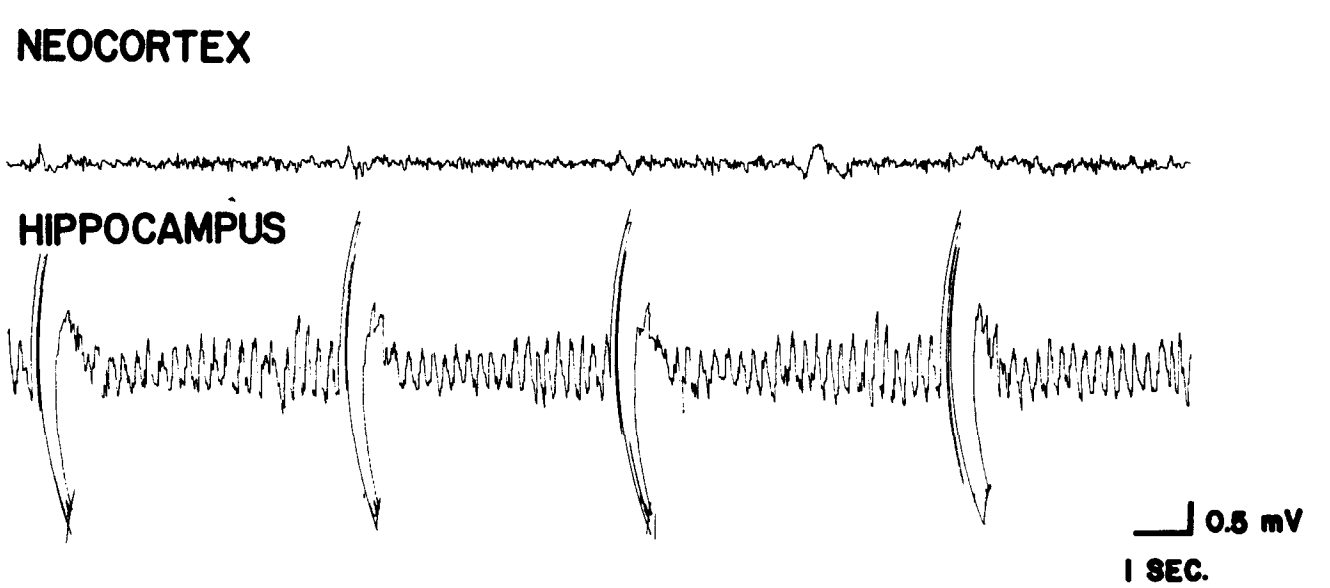

FIG. 4. Neocortical and hippocampal EEG in an eserinized rat 10 min after receiving 500 μg of atropine sulfate in the ventricle. Note the large spikes which occur periodically in the hippocampal record. Such spikes were typical of the hippocampal record of animals treated with doses of atropine exceeding 200 μg. Note also that RSA is still present. The animal was lying down and movement, with the exception of twitches, was absent.

Other Effects of Intraventricular Anti-cholinergic Agents

Despite the fact that intraventricular anti-cholinergics did not abolish RSA, vasodilation of the ears of rabbits was quite obvious after injections of 50–200 μg. In the experiments with urethane, slight pupillary dilation followed 50 μg of atropine and increased with each subsequent injection until dilation was maximal after 200 μg.

DISCUSSION

The experiments show that immobility-related neocortical desynchronization and hippocampal RSA are blocked by systemic atropine and scopolamine. Mobility-related neocortical desynchronization and hippocampal RSA were not blocked by systemic atropine or scopolamine. These results support previous findings that there are two types of cerebral activation, one which is anti-cholinergic sensitive, the other which is anti-cholinergic resistant [18, 28, 30]. In contrast to the effects of systemically administered anti-cholinergic agents, intraventricular injections of atropine and scopolamine blocked neither type of EEG activity in the neocortex or hippocampus. The results confirm a previous report that intraventricular atropine (300–500 μg) given to the cat failed to produce the neocortical slowing obtained with systemic injections [6]. We interpret the results as suggesting that atropine and scopolamine do not penetrate to the cholinergic neural target as effectively from the ventricular fluid as from the capillary bed. The results also suggest that EEG recording techniques can be used effectively to assess penetration of pharmacological substances given by different routes.

The failure of central injections to block anti-cholinergic sensitive EEG activity does not seem attributable to procedural variables. Histological evidence showed that the cannulae were successfully located in the ventricles. Further, the wide distribution of the injected dye suggested that the drugs should have been distributed throughout the fluid spaces. The drug dosage levels also seemed sufficiently large. From Tønnesen's [26] results it can be estimated that 6 μg of atropine evenly distributed throughout the brain would be sufficient to block anti-cholinergic sensitive EEG activation in the rat. In the present study dosage levels were 30–80 times this large. Also, the dosage levels were well within ranges of intraventricular injections used by other workers to influence behavioral or phsyiological events [10, 15, 24].

It is unlikely that the EEG blocking action of anti-cholinergic agents delivered via a systemic route is mediated peripherally rather than centrally. The quaternary ammonium compound, atropine methyl nitrate, does not cross the blood-brain barrier and does not block cerebral patterns of EEG activity, although it is somewhat more potent than atropine sulfate [16]. Further, atropine sulfate has an effective action in the *cerveau isolé* (a preparation in which all peripheral input is blocked), suggesting that atropine acts at the mesodiencephalic junction or higher [6,22].

The central target of the anti-cholinergic agents is not known but the problem has received attention. Although transection studies have shown that atropine is effective in the *cerveau isolé* preparation, it is no longer effective after hemispheric transection [6,22]. Thus, it has been suggested that atropine acts at the level of the mesodiencephalic junction and not diffusely on cortical structures [6,22]. However, neocortical desynchronization and hippocampal RSA survive massive destruction to mesodiencephalic areas [23] and these postoperative EEG patterns have been found to be anti-cholinergic sensitive (Whishaw, unpublished data). Therefore, the location of the anti-cholinergic sensitive neural system remains to be determined. For the present it may be suggested that intraventricular anti-cholinergic agents were ineffective because either: (a) there is an effective cerebrospinal fluid-brain barrier to the substances tested, (b) the target is distant from the ventricles and possibly quite diffuse [19,25], or (c) the

target is insulated by white matter through which drug diffusion has been described as slow or negligible [9].

Bilateral decreases in RSA amplitude were recorded in rats given intraventricular anti-cholinergic agents during urethane anaesthesia. It is possible that the anti-cholinergics reduced RSA amplitude by acting on one of the two RSA generators [5]. The CA-1 generator seems a likely candidate since it is superficial to the ventricular surface. Any such action could be a result of the blocking action of anti-cholinergics on the activity of cells in CA-1 which have been described as sensitive to iontophoretically applied atropine [4] or due to local anaesthetic action on neural tissue [7]. However, if the anti-cholinergics acted on the hippocampus directly they should have had an equivalent action in the eserinized rats. This was not the case. Another possible explanation of the RSA amplitude decrease in the urethanized rats is that the anticholinergic agents augmented the anaesthetic properties of urethane. We did observe in preliminary experiments that if urethane doses were too large hippocampal RSA amplitude could be profoundly depressed.

We also observed in the experiments with urethane that neocortical desynchronization could still be obtained after intraperitoneal atropine treatment. Neocortical desynchronization is not seen after combined treatments of ethanol and atropine in rabbits [30] or after combined treatments of ether and atropine in rats [28]. All of the drug treatment combinations do block RSA which suggests that urethane has an action on the neocortex which is different from ethanol and ether and also is different from the action of urethane on the hippocampus. This indicates that there are differences in the systems which mediate desynchronization in the neocortex and RSA in the hippocampus.

Finally, it is important to note that high doses of atropine given centrally (>200 μg) produce hippocampal electrographic abnormalities such as spiking which do not occur following intravenous and intraperitoneal injections. This type of effect should be considered in studies in which atropine or other drugs are given centrally and suggests that brain EEG activity should be monitored during injection. The results also suggest that electrical recording procedures can serve as a useful indication of the action of a drug given by different routes. In the present experiments it was demonstrated quite clearly that the actions of centrally injected anti-cholinergic agents do not parallel the actions of the drugs when they are administered systemically.

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