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Effects of some sympathomimetic drugs and their antagonists on afterdischarges elicited in chronically isolated slabs of cerebral cortex

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Summary

1. The role of sympathomimetic agents in the maintenance and termination of induced cortical epileptiform activity was studied in chronically neuronally isolated slabs of cerebral cortex in the suprasylvian gyrus of unanaesthetized, unrestrained cats.

2. The administration of the sympathomimetic agents (+)-amphetamine, methamphetamine, tyramine, and ephedrine resulted in a highly significant decrease in the duration of epileptiform afterdischarge (EADs).

3. The α-adrenoceptor blocking drugs phenoxybenzamine, phentolamine and tolazoline did not significantly alter the duration of EADs but prevented the decrease in duration of EADs produced by the sympathomimetic drugs.

4. The effect of atropine and arecoline on the duration of EADs, previously described, were not modified by the α-adrenoceptor blocking drugs, but atropine prevented and reversed the inhibitory action of amphetamine.

5. It is suggested that (1) in the chronically neuronally isolated cortical slab there is normally no spontaneous adrenergic activity, (2) a cortical, cholinergic inhibitory mechanism, previously described, is modulated by ascending adrenergic influences, (3) adrenergic cholinergic linkages might be arranged in the cortex in an alternating network, as proposed by Feldberg.

Introduction

Chronic neuronal isolation of an area of cerebral cortex results in an increased tendency of the isolated region to respond with long-lasting epileptiform afterdischarges (EADs) to direct electrical stimulation (Graftstein & Sastry, 1957; Sharpless & Halpern, 1962). The neuronally isolated cortical slab may constitute a suitable model for the study of grand-mal epilepsy (Sanders & Gravlin, 1968; Krip & Vázquez, 1970). It has the advantage that the isolated tissue is devoid of subcortical influences, and can thus be used to study the cortical mechanisms involved in seizure activity.

We have previously postulated (Vázquez, Krip & Pinsky, 1968, 1969) the presence of a cortical cholinergic inhibitory system, responsible for the termination of epileptiform activity in chronically isolated cortical slabs. The cholinergic drugs pilocarpine, arecoline, eserine and oxotremorine significantly decreased the duration of

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EADs and this action was antagonized by atropine or scopolamine. This action seemed to be centrally mediated, and not due to the peripheral cardiovascular actions of these agents, because neither methylatropine nor methacholine produced any change in the duration of EADs (Vázquez *et al.*, 1969 ; Krip & Vázquez, 1970).

Livingston, Kajdi & Bridge (1948) and Livingston (1966) have reported on the antiepileptic action of amphetamines and suggested that aside from their electroencephalographic synchronizing ability, they act through specific adrenergic sites involved in seizure control. In contrast to these results Minz & Domino (1953) found that sympathomimetic agents prolong the duration of cortical afterdischarges in spinal cats.

Rudzik & Mennear (1966) studied the mechanisms of action of anticonvulsants in mice and found that prior administration of reserpine or adrenoceptor blocking agents prevented the anticonvulsant activity of acetazolamide. Green, Halpern & Van Niel (1970) have shown that monoamine oxidase (MAO) activity in chronically isolated slabs increased by 55% after 2 weeks of isolation. These authors speculate that this should then result in a deficiency of adrenergic transmitter. If the adrenergic transmitter has an inhibitory function in the cortex as has been suggested by Salmoiraghi (1966), this increase in MAO activity in isolated slabs would result in a state of hyperexcitability and the administration of sympathomimetic drugs should overcome this tendency towards hyperexcitability.

Pepeu & Bartolini (1968) have shown that the administration of amphetamine increases cortical acetylcholine (ACh) output by 70%. This increase in ACh output is associated with EEG activation and an increase in blood pressure. More recently, the same authors (Bartolini & Pepeu, 1970) concluded that this increase in ACh output by amphetamine was probably due to a central action on β-adrenoceptors, because pretreatment with the β-adrenoceptor blocking agents pronethalol and propranolol completely blocked the increase in central ACh output after amphetamine administration, but did not block the pressor response. On the other hand, pretreatment with phenoxybenzamine prevented the hypertensive action of amphetamine but did not prevent the increase in cortical ACh output. These authors concluded that there are central β-adrenoceptors where the catecholamines could act to stimulate cholinergic neurones.

It seemed reasonable, therefore, to examine the role of sympathomimetic agents in the maintenance and termination of cortical epileptiform activity in neuronally isolated cortical slabs, and to study their interactions with the cortical cholinergic inhibitory system that we previously postulated (Vázquez *et al.*, 1969). A preliminary report of the findings was presented to the American Society for Pharmacology and Experimental Therapeutics in August 1970.

Methods

Cats (2·5–4·0 kg) of either sex, were anaesthetized with pentobarbitone sodium (35 mg/kg i.p.). The dorsal aspect of the suprasylvian gyrus on the left hemisphere was exposed by craniotomy of an appropriate area of parietal bone. A slab of cerebral tissue 5 mm × 15 mm × 3 mm deep was then isolated by techniques previously described (Vázquez *et al.*, 1969). This provided a slab of cerebral cortex, completely free of all neuronal connexions with adjacent non-isolated brain, but preserving the pial and arachnoidal blood vessels intact.

An electrode assembly consisting of eight electrodes (platinum–10% iridium) terminating in a small plastic socket, was chronically implanted over the operated gyrus, by a modification of the technique described by Sharpless & Halpern (1962). Six of the electrodes were confined within the margins of the slab while the remaining two rested on the surface of adjacent non-isolated cortex. A schematic diagram of this electrode arrangement is included in Fig. 1. The cats were allowed to recover and were kept for repeated experiments for up to 6 months.

For stimulating and recording, the cats were placed within an observation box (1 m on edge) which permitted them to move freely and be observed. The socket in which the electrodes terminated was connected to a flexible cable which led to the recording and stimulating system. Signals from the electrodes were amplified by RC-coupled amplifiers (Grass, P5) and recorded on an ink writing polygraph (Grass, 5C).

Recording and stimulation were bipolar. One pair of the electrodes resting within the isolated slab was selected to apply 3 ms monopolar rectangular pulses at a rate of 40 Hz. Pulse trains lasted from 2 to 5 s as necessary to produce EADs. The remaining pairs of electrodes were used to monitor the electrical activity from the isolated and the non-isolated cortex.

Threshold stimulus intensity (assumed to be proportional to pulse amplitude) was determined individually during each session by increasing pulse amplitude (at 20 min intervals) until an EAD was elicited. The experiments were then carried out with a voltage 10% greater than the threshold. Between stimulus trains the cats were rested for 20 min because previous work in this laboratory (Vázquez *et al.*,

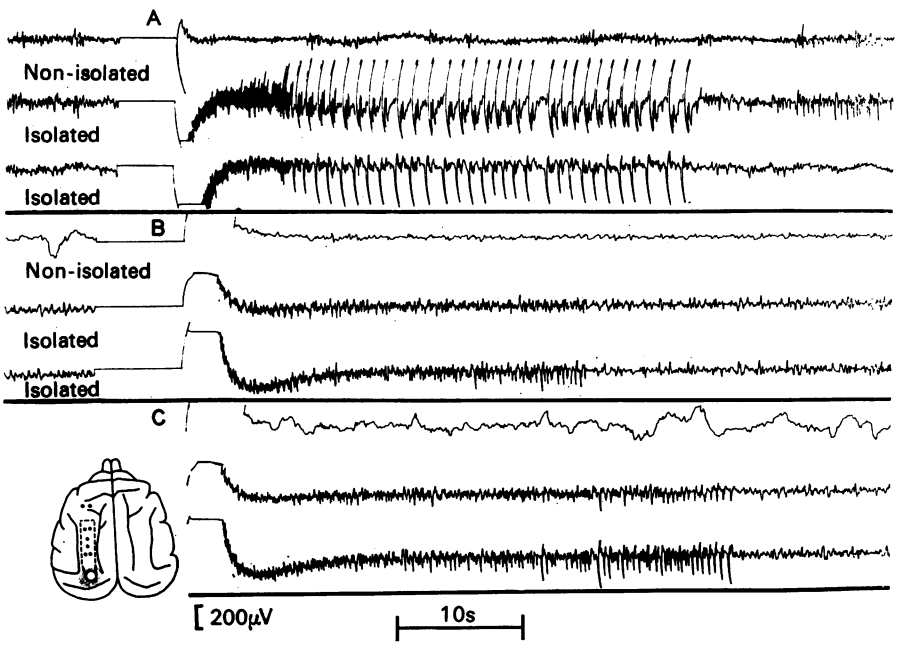

FIG. 1. Examples of afterdischarges elicited in a chronically isolated slab from three different cats (A, B, C) stimulated at regular weekly intervals. The average duration of EADs in these preparations is approximately 35 seconds. The diagram in the lower left-hand corner represents the position of the isolated cortical slab and the electrode arrangement. Isolated: record from electrodes within the slab. Non-isolated: record from the pair of electrodes outside the isolated slab. Note the abrupt cessation of the EAD.

1969) has shown that the afterdischarge is subject to significant fatigue and variability if the interstimulus interval is less than 15 minutes.

Testing was begun at least 1 week after the surgery and implantation procedures. Preliminary control experiments were carried out on each animal to establish the reproducibility of the effect of repeated afterdischarges on untreated isolated slabs during a period which would correspond in time to an experimental session.

After the threshold was determined, three consecutive and stable EADs were elicited at voltages and intervals as described above. At this point saline (or the drug-free vehicle) was injected intraperitoneally as a control, and the slab was again stimulated 3 times using the same parameters. The drug under study was then administered and the slab stimulated again in the same manner. Subsequently the drug was injected after the control responses were elicited.

Afterdischarge duration was measured from the point of withdrawal of the stimulus to the point of abrupt termination of paroxysmal 'convulsive' spiking activity. As can be seen in Fig. 1 there is usually a sharp transition from epileptiform activity to resting activity. In each experiment EAD durations after drug treatment were compared with control durations by means of Student's t test (Steel & Torrie, 1960). In each drug series (composed of at least six different cats) the mean control and 'treated' EAD durations and their standard errors of the mean were calculated for the control and drug treatments. The mean differences in EAD duration from control were then calculated from the appropriate set of control and

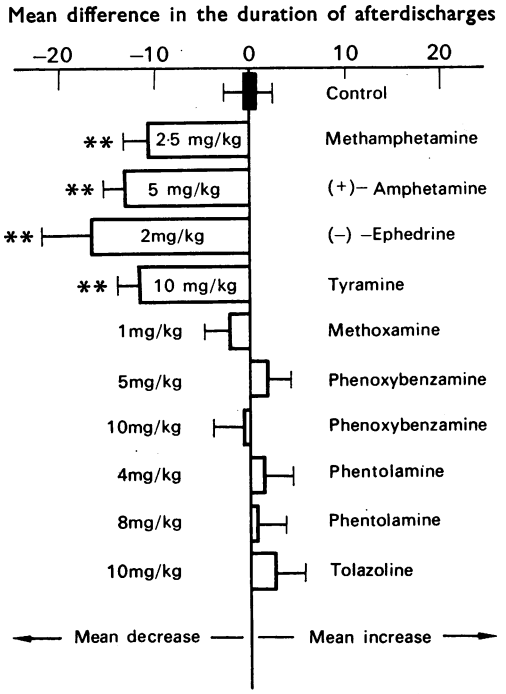

FIG. 2. Effect of sympathomimetic agents and antagonists upon the duration (in seconds) of EADs elicited in chronically isolated slabs. Each bar represents the mean of the differences ±S.E. between the control and 'after-drug' duration of EAD. Each bar is the mean of at least six experiments at each dose level, except for tolazoline and methoxamine where the bar represents four experiments. $^{**}P \lesssim 0{\cdot}01$.

drug treated measurements. The present report is based on 120 experiments in sixteen cats.

The cats were rested for at least 7 days after an experiment with any given drug. Each cat was tested with approximately eight different drugs. The duration of EADs was continuously monitored for the first 4 h, and then again at 24 h, after drug administration.

The effects of the following drugs upon the duration of EADs were investigated: methamphetamine hydrochloride (2·5 mg/kg), (+)-amphetamine sulphate (5 mg/kg), (−)-ephedrine sulphate (2 mg/kg), tyramine hydrochloride (10 mg/kg), methoxamine hydrochloride (0·25 mg/kg, 1 mg/kg), phenoxybenzamine hydrochloride (5 mg/kg, 10 mg/kg), phentolamine hydrochloride (4 mg/kg, 8 mg/kg), tolazoline hydrochloride (10 mg/kg), atropine sulphate (3 mg/kg) and arecoline hydrochloride (1 mg/kg). All drugs were dissolved in normal saline or distilled water (injection volume 1 ml/kg or less) except for phenoxybenzamine, for which it was necessary to use acidified-propylene glycol (injection volume 0·2 ml/kg). All drugs were administered intraperitoneally approximately 5 min before stimulation.

Results

Effect of sympathomimetic drugs and antagonists upon the duration of EAD

The results of the studies done with seven different drugs are summarized in Fig. 2. The average control duration of EADs in these experiments was 35 s, which closely agrees with our previously reported results (Vázquez *et al.*, 1969).

All the centrally active sympathomimetic agents tested (Fig. 2), methamphetamine hydrochloride (2·5 mg/kg), (+)-amphetamine sulphate (5 mg/kg), (−)-ephedrine

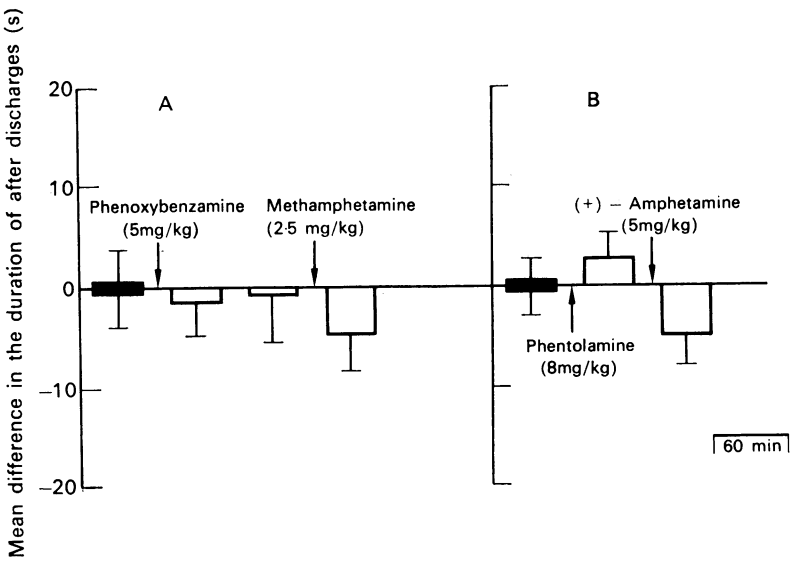

FIG. 3. Antagonism by α-adrenoceptor blocking agents of the effects of sympathomimetic agents upon the duration of EADs. Each bar represents in six cats the mean ± S.E. of the differences in duration between three consecutive EADs and the mean control duration in the same cat. EADs were elicited at 20 min intervals. Drugs were injected intraperitoneally at the time indicated by the arrows. Note that the α-adrenoceptor blocking agents prevent any significant action of the sympathomimetic drugs upon EAD.

sulphate (2 mg/kg), and tyramine hydrochloride (10 mg/kg), significantly ($P \leq 0{\cdot}01$) decreased the duration of afterdischarge. The duration of EADs had returned to control values 24 h after drug administration.

The peripheral effects (piloerection, tachycardia) of these agents were evident (except for tyramine) 10 min after injection, and persisted for at least 2 hours. In the case of tyramine, effects were not evident until approximately 30 min after drug injection. All the cats showed some behavioural signs.

Methoxamine hydrochloride, a sympathomimetic agent devoid of central action (Innes & Nickerson, 1970) was also tested (Fig. 2). Five minutes after injection, peripheral signs of adrenoceptor stimulation were evident. However this agent did not modify the duration of EAD.

The α-adrenoceptor blocking agents, phenoxybenzamine hydrochloride (5 and 10 mg/kg), phentolamine hydrochloride (4 and 8 mg/kg) and tolazoline hydrochloride (10 mg/kg) had no significant effect upon the duration of EADs (Fig. 2). Within 20 min of the intraperitoneal injection of these agents their peripheral effect was evident (relaxation of the nictitating membrane). The cats showed detectable behavioural depression and appeared slightly drowsy and inactive. The responses to these agents were monitored for over 4 h with the slab stimulated at 20 min intervals, which would seem to allow enough time for the drug to block central adrenoceptors. Except for phenoxybenzamine, the peripheral effects of the α-adrenoceptor blocking agents disappeared within 150 minutes. The effect of these agents upon the duration of EADs was also tested in a few animals at lower doses (phenoxybenzamine hydrochloride 2·5 mg/kg and phentolamine hydrochloride 2 mg/kg) which were likewise devoid of any significant effect upon EAD duration.

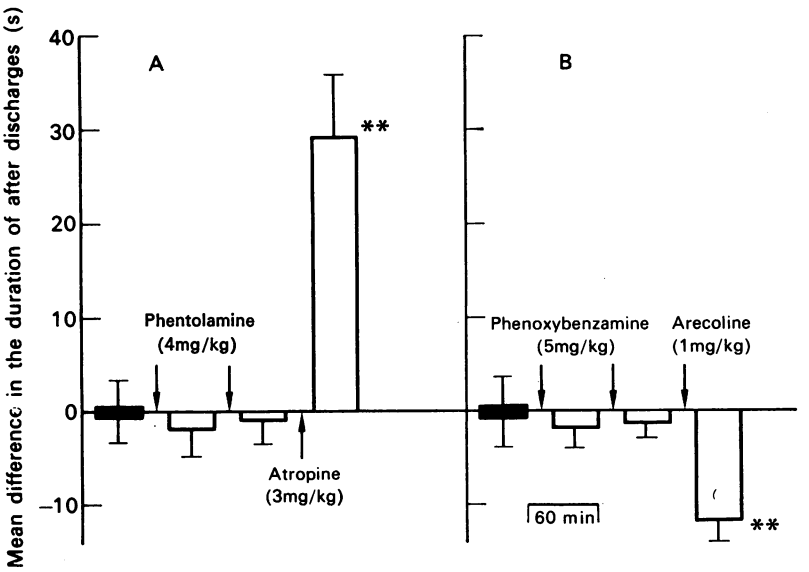

FIG. 4. Lack of antagonism by α-adrenoceptor blocking agents of the effects of acetylcholine-like drugs upon the duration of EADs. Each bar represents in six cats (A) and eight cats (B) the mean ± S.E. of the differences in duration between three consecutive EADs and the mean control duration in the same cat. EADs were elicited at 20 min intervals. Drugs were injected intraperitoneally at the time indicated by the arrows. Note that the α-adrenoceptor blocking agents do not prevent atropine or arecoline from exerting a significant change in the duration of EADs. $^{**}P \leq 0{\cdot}01$.

Two series each of six experiments were conducted to determine if pretreatment with α-adrenoceptor blocking drugs would prevent the decrease in duration of after-discharge produced by sympathomimetic agents (Fig. 3). Phenoxybenzamine (5 mg/kg) administered 90 min before the injection of methamphetamine hydrochloride (2·5 mg/kg) prevented methamphetamine from producing any significant alteration in EAD duration. Methamphetamine consistently (six out of six experiments—Fig. 3A) produced a small decrease in the duration of EADs, but this decrease was not significant when analysed by the *t* test. In another similar series of experiments, pretreatment with phentolamine hydrochloride (8 mg/kg) prevented the action of amphetamine upon the duration of EADs (Fig. 3B).

Interaction between adrenoceptor blocking agents and acetylcholine-like drugs and their antagonists

In six experiments (Fig. 4A) the administration of 3 mg/kg of atropine sulphate produced a significant ($P \leq 0{\cdot}01$) increase in the duration of EADs. Pretreatment with phentolamine hydrochloride (8 mg/kg) did not prevent this action of atropine. The increase in duration produced by atropine is of the same order of magnitude as in animals that have not been pretreated (Vázquez *et al.*, 1968, 1969). In another series of eight experiments, pretreatment with phenoxybenzamine (10 mg/kg) (Fig. 4B) did not prevent the decrease in EAD duration produced by arecoline (1 mg/kg). This decrease in duration is significant ($P \leq 0{\cdot}01$) and of the same order of magnitude as the decrease in EAD duration produced by arecoline in animals that have not been pretreated with any drug (Vázquez *et al.*, 1968, 1969).

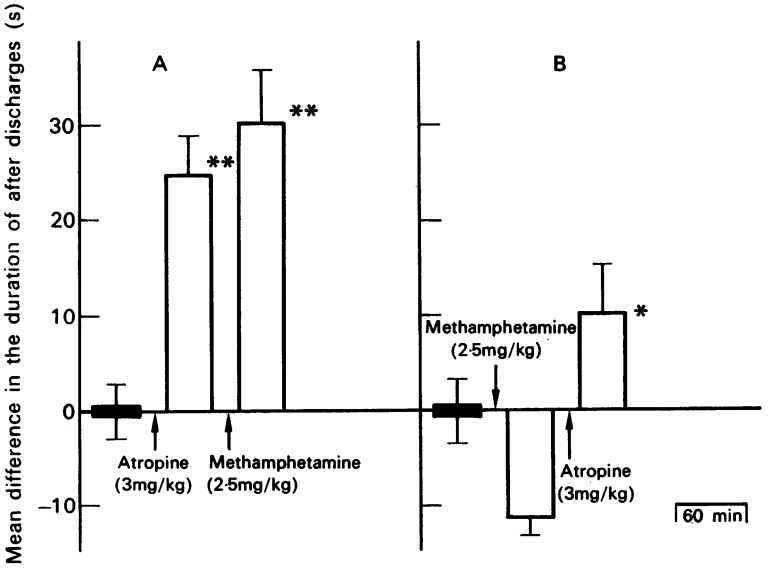

FIG. 5. Interactions between atropine and methamphetamine. Each bar represents in eight cats the mean ± S.E. of the differences in duration between three consecutive EADs and the mean control duration in the same cat. EADs were elicited at 20 min intervals. Drugs were injected intraperitoneally at the time indicated by the arrows. A, Note that methamphetamine does not modify the increase in duration of EADs produced by atropine. B, Atropine reverses the decrease in the duration of EADs produced by methamphetamine. If the duration of EADs after amphetamine is used as a baseline to measure the increase in duration of EADs produced by atropine, this increase becomes highly significant ($P \lesssim 0{\cdot}01$) and is of the same magnitude as that produced by atropine in Figs. 4A and 5A. $*P \lesssim 0{\cdot}05$, $**P \lesssim 0{\cdot}01$.

Interactions between atropine and sympathomimetic agents

In eight experiments, the administration of atropine sulphate (3 mg/kg) produced a significant increase in the duration of EADs and completely blocked the effect of a subsequent dose of methamphetamine hydrochloride (2·5 mg/kg) (Fig. 5A). In another series of eight experiments, methamphetamine (2·5 mg/kg) produced a highly significant ($P \leq 0{\cdot}01$) decrease in the duration of EADs. This decrease was antagonized and reversed by a subsequent injection of atropine (3 mg/kg) (Fig. 5B). The mean increase in duration of EADs resulting from the administration of atropine was 11 s, with respect to control values. However, as methamphetamine had decreased the mean duration of EAD by 12 s, the mean total increase in the duration of EADs produced by atropine was 23 seconds. This value is approximately equal to the increase in duration of EADs produced by atropine in animals that have not received any pretreatment.

Discussion

In this study, all the centrally active sympathomimetic drugs used significantly decreased the duration of EADs. This is an apparent contradiction of the results of Minz & Domino (1953), who, however, did not study the EAD in isolated cortical slabs but in spinal cats, where actions of the drugs upon brain structures other than the cortex might be reflected in the EAD. Furthermore, an acute heparinized spinal experimental preparation has been subjected to considerable trauma with the concomitant release of cerebroactive substances, and the action of sympathomimetic drugs (given intravenously or by intra-arterial injection) might not necessarily reflect the changes in the measured parameters to be expected under conditions of less immediate trauma. The fact that methoxamine, a sympathomimetic drug apparently lacking central actions (Innes & Nickerson, 1970) had no effect upon the duration of EADs, suggests that the decrease in duration of EADs produced by the sympathomimetic drugs used is due to an action of these agents upon central structures, and not secondary to the cardiovascular changes induced by them. We cannot ascertain, on the basis of these results, whether the effects observed are the result of actions on adrenoceptors or whether the sympathomimetic agents used act through other types of receptors. Vane (1960), has considered the possibility that the central action of very high doses of amphetamine might be mediated via 5-hydroxytryptamine receptor sites. However, we are inclined to implicate in this action upon the duration of EAD the participation of some of the cortical adrenergic structures (Dahlström, 1969; Johnson, Roberts, Sobieszek & Straughan, 1969), because not only amphetamine, but ephedrine and tyramine, which are not suspected of stimulating 5-hydroxytryptamine receptors, also significantly decreased the duration of EADs.

α-Adrenoceptor blocking agents antagonize significantly the effect of sympathomimetic drugs upon the duration of EADs, which suggests that the adrenoceptors involved in the modulation of epileptiform activity are of the α type.

The adrenoceptor blocking agents studied did not themselves produce any significant change in the duration of EADs. Their lack of effect cannot be attributed to poor penetration in the central nervous system. Phenoxybenzamine is a highly lipid soluble substance (Brodie, Aronow & Axelrod, 1954) and there is evidence of its central effects (Muñoz & Goldstein, 1961); there is also evidence that phentolamine penetrates through the blood-brain barrier and has effects on the central

nervous system (Brodie, Spector & Shore, 1959). The lack of effect upon the EAD of the adrenoceptor blocking agents can only be explained by assuming that in the isolated slab there is no adrenergic tone. The adrenergic junction if present, therefore, may be of the 'potential' type described by Sabelli (1964). Alternatively the cell body of the adrenergic neurone may be located below the level of the cortex. In that case the chronic isolation procedure would damage the presynaptic neurone, effectively denervating the adrenoceptor, and only agonists acting at the postsynaptic site would still be able to alter the duration of EADs. All the sympathomimetic drugs used are direct acting to a certain degree (Trendelenburg, Muskus, Fleming & de la Sierra, 1962). As Fig. 2 shows, the most effective agent in our experiments was ephedrine, the centrally active sympathomimetic agent with more significant direct sympathomimetic actions (Nickerson, 1970) of the series used. This is consistent with, but not proof of, the idea that we may be studying a denervated adrenoceptive structure.

The observed interactions between atropine and the sympathomimetic agents used, suggest that the decrease in duration of EADs which the adrenergic sympathomimetic drugs produce is mediated through stimulation of cholinergic neurones. Atropine prevents the action of amphetamine, but pretreatment with α-adrenoceptor blocking agents does not prevent the decrease in duration of EADs produced by cholinomimetics. There is evidence that amphetamine increases cortical ACh release (Pepeu & Bartolini, 1968; Beani, Bianchi, Santinoceto & Marchetti, 1968; Hemsworth & Neal, 1968) and that this increase in release can be prevented in the rat by α-adrenoceptor blocking agents (Hemsworth & Neal, 1968). Bartolini & Pepeu (1970) were able to prevent the increase in ACh release produced by amphetamine in the cat with β-adrenoceptor blocking agents only, while with α-adrenoceptor blocking agents which they used did not modify cortical ACh output. Current histochemical evidence (Dahlström, 1969) indicates that there are noradrenergic neurones with cell bodies mainly found in the pons and the medulla oblongata and axons that ascend close to the medial forebrain bundle and innervate the cortex. These neurones may very well synapse with the neurones involved in the cortical cholinergic inhibitory system previously described (Vázquez, *et al.*, 1969). Excitation of the adrenergic neurones would activate the cholinergic inhibitory system.

The presence of such an adrenergic-cholinergic alteration in the central nervous system, had already been proposed by Feldberg (1950) and might explain why atropine blocks the EEG effects of sympathomimetic amines (Toman & Davis, 1949; White & Daigneault, 1959; Longo, 1962), but not their behavioural effects.

In conclusion, the results which have been presented here are consistent with the hypothesis that the activity of the cortical cholinergic inhibitory mechanism (Vázquez, *et al.*, 1969) involved in EAD control, is modulated by adrenergic neurones whose cell bodies are not located in the cortex.

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