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EFFECTS OF DRUGS WHICH DEPRESS THE PERIPHERAL NERVOUS SYSTEM ON THE RETICULAR ACTIVATING SYSTEM OF THE CAT

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Some drugs with depressant properties on the peripheral nervous system have been examined for depressant effects on the reticular activating system of the cat. Large doses of nicotine, or of the anti-nicotinic agents dihydro-β-erythroidine and mecamylamine, failed to depress the reticular activating system; non-quaternary drugs with anti-muscarinic properties, such as atropine and hyoscine, depressed it readily. Hyoscine was the most potent depressant tested and its effects could be antagonized by physostigmine. In contradistinction, depression of the reticular activating system with pentobarbitone was not antagonized by physostigmine. Lignocaine was a weak depressant of the reticular activating system, and the possibility that this might be due to a central anti-muscarinic action is discussed. Adrenergic blocking drugs, such as dihydroergotamine, phenoxybenzamine or choline 2 : 6-xylyl ether, did not appear to depress the reticular activating system: the significance of this is discussed. It was concluded that the hypothetical cholinergic transmitter, acting somewhere within the reticular activating system, displayed actions analogous to the muscarinic, and not to the nicotinic, actions of acetylcholine.

The discovery by Moruzzi and Magoun (1949) that electrical stimulation of the brain stem reticular formation is followed by behavioural and electroencephalographic signs of arousal in animals was an outstandingly important contribution to neurological science. The recognition of such a reticular activating system has provided neurophysiologists, and in particular Magoun and his associates, with a means of disentangling some of the complex reactions of the brain to the influx of sensory information; it has stimulated neuroanatomists in their search for recognizable functional pathways inter-connecting cortex and midbrain; and it has given neuropharmacologists a valuable tool for investigating the effects of drugs upon the elusive central synapse.

The demonstration that the reticular formation is particularly susceptible to the depressant effects of hypnotic and anaesthetic agents (French, Verzeano, and Magoun, 1953a; Arduini and Arduini, 1954) has been followed by several useful analyses of the effects of various drugs on the reticular arousal system (for example, Domino, 1955; King, 1956; Gangloff and Monnier, 1957; Bradley and Key, 1958; Kuehn and Schallek, 1958). Other workers, notably Bradley and Elkes (1957), have studied the effects of drugs on the state of arousal of unrestrained, unanaesthetized animals, and have provided further indirect evidence of possible drug influences on the reticular system.

Evidence to date suggests that cholinergic neurones play an important rôle in the reticular activating system (Rinaldi and Himwich, 1955a, 1955b) and are hence to be considered along with other factors determining changes in the electrical pattern recorded from the cortex itself. There is no doubt that an adrenaline-sensitive component also exists within the reticular activating system (Bonvallet, Dell, and Hiebel, 1954; Rothballer, 1956).

In the present work some drugs which are known to depress the peripheral nervous system have been examined for possible depressant actions on the reticular activating system of the cat.

METHODS

Medium-sized cats were anaesthetized with ether and a tracheotomy tube inserted. A polythene tube for the injection of drugs was secured in the right femoral vein. Blood pressure was monitored

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routinely by a modified anaeroid gauge, carefully calibrated, which was connected to a polythene tube in the right femoral artery.

The skull was partly exposed and cortical potentials recorded on a Grass Model IV-A electroencephalograph; gramophone-needle electrodes (Hoagland, 1940) were inserted through the posterior walls of the frontal sinuses and dura (in order to make contact with the sensorimotor areas), and through burr holes drilled almost down to the dura over the marginal gyri. Electrocardiograms were recorded from needle electrodes placed in the forelimbs.

The head was fixed in a Lab-Tronics Model 4 stereotaxic instrument and a concentric stimulating electrode, constructed according to Jasper and Droogleever-Fortuyn (1947), was introduced through a hole in the skull and dura until its tip was positioned at Horsley-Clarke co-ordinates F+2, L 3, H−1. These were chosen after reference to the stereotaxic atlas of Jasper and Ajmone-Marsan (1954). The electrode tip was never moved outside a radius of 1 mm. from the selected point. In view of the consistently low stimulus threshold-voltage for electroencephalographic activation required at this point, and the latitude afforded by the relatively large area of reticular substance at this frontal plane, the placement of the electrode was not confirmed histologically as a routine.

At the end of the surgical procedures the external auditory meati and all wounds were infiltrated with 1% procaine solution; ether anaesthesia was discontinued, respiration supported artificially, and the preparation immobilized with serial injections of tubocurarine. Body temperature was maintained by infra-red radiation. The room was darkened and kept as quiet as possible in order to encourage drowsy or sleep-like electroencephalographic patterns.

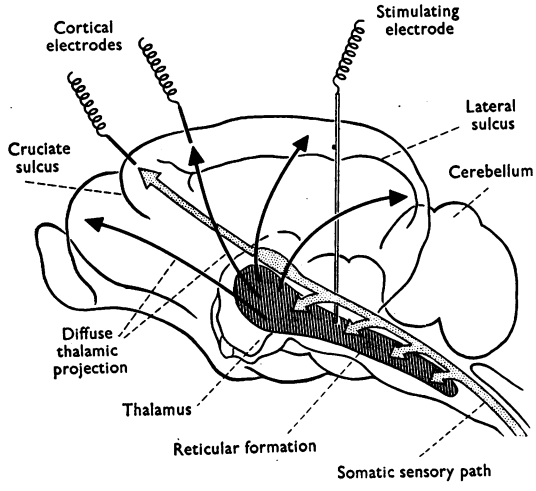

FIG. 1.—Diagrammatic representation of lemniscal and extralemniscal afferent systems radiating to cat cerebral cortex; approximate relation of stimulating and recording electrodes to these structures is shown.

After 2 hr., when the effects of ether had worn off, the treatment with local anaesthetic was repeated. The reticular formation was stimulated (not more than once every 3 min.) for 3 sec. periods with supramaximal rectangular pulses (6 V., 0.1 msec. duration, 250 shocks/sec.) through a Grass stimulator and isolation unit. One channel of the electroencephalograph was utilized for stimulus marking.

Observations on the arousal responses to sensory stimulation were made from time to time by stroking the back of the animal firmly with a glass rod.

Fig. 1 shows, in a diagrammatic form, the brain stem reticular formation, the diffuse projection from thalamus to cortex, the somatic sensory path and the sensory collateral connexions (Starzl, Taylor, and Magoun, 1951; French, Verzeano, and Magoun, 1953b) leading into the reticular formation. The approximate relation of stimulating and recording electrodes to these structures is also shown.

RESULTS

Electroencephalographic Response to Reticular Stimulation

Typical electroencephalographic activation changes are shown in Fig. 2, firstly in response to a 3 sec. period of electrical stimulation of the reticular formation, and secondly in response to touch.

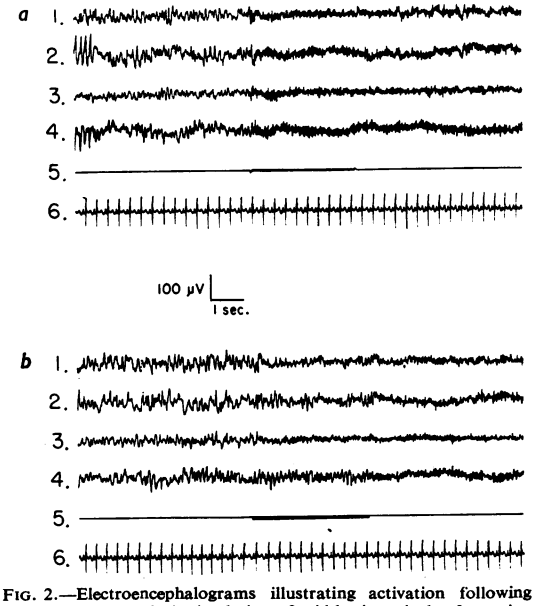

FIG. 2.—Electroencephalograms illustrating activation following: *a*, 3 sec. electrical stimulation of mid-brain reticular formation; *b*, tactile stimulation (stroking skin of back with glass rod). Note replacement of spontaneous slow potentials by low-voltage fast activity during and following each stimulus. Channels: **1**, left post. cruciate gyrus to left marginal gyrus; **2**, right post. cruciate gyrus to right marginal gyrus; **3**, left post. cruciate gyrus to right post. cruciate gyrus; **4**, left marginal gyrus to right marginal gyrus; **5**, stimulus marker; **6**, electrocardiogram.

The activated electroencephalographic pattern, the so-called "desynchronized," "asynchronous," or "alerted" pattern which is usually associated with behavioural arousal, is characterized by low-voltage fast activity (30 to 50 c./sec.) with reduction in voltage of the slow (1 to 10 c./sec.) waves seen in the drowsy or sleeping animal and, of course, an absence of sleep spindles. It must be realized, however, that various gradations in electroencephalographic pattern exist between the extremes of sleep and full arousal as has been emphasized by Rothballer (1956). Moreover, drugs may sometimes produce electroencephalographic patterns which simulate those of sleep or full arousal without corresponding overt behaviour (Wikler, 1952 ; Bradley and Elkes, 1957).

The drugs examined have been divided into groups according to their effects on other parts of the nervous system.

Anti-muscarinic Agents

Hyoscine Hydrobromide.—This is the most potent depressant of reticular activation of the electroencephalogram which we have tested. Fig. 3*a* shows part of a record taken about 5 min. after the intravenous injection of 0.1 mg./kg. of hyoscine. Cortical potentials were invariably slowed in frequency by the drug, and sleep spindles usually appeared. The almost complete abolition of the response to reticular stimulation will be noted. Tactile stimulation was likewise ineffective in promoting activation.

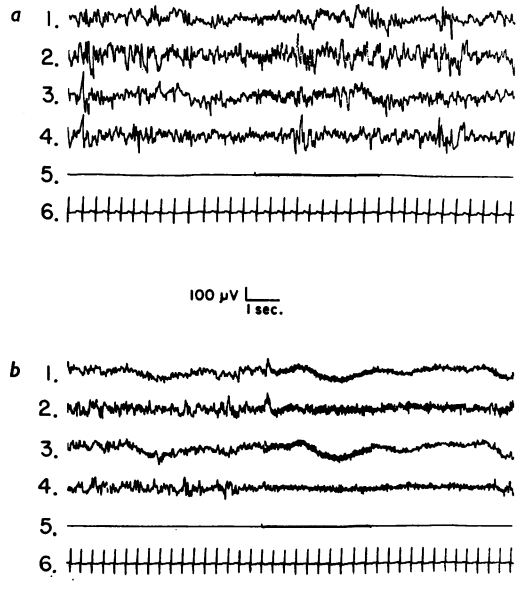

FIG. 3.—Electroencephalograms, channels as in Fig. 2. *a*, Failure of electroencephalographic activation by reticular stimulation 5 min. after giving intravenous hyoscine hydrobromide (0·1 mg./kg.); *b*, reversal of this hyoscine-block of reticular activating system by subsequent dose of physostigmine sulphate (0 2 mg./kg.).

Atropine Sulphate.—Compared with hyoscine, we found atropine to be relatively weak as a depressant of reticular activation. Doses exceeding 0.4 mg./kg. produced slowing of the spontaneous cortical potentials. In the dose range 2 to 10 mg./kg., activation occurred only during the period of application of the electrical stimulus ; even after 10 mg./kg., complete block of reticular activation, such as was seen with hyoscine, did not occur ; the response to tactile stimulation was, however, abolished at this dose.

Methanthelinium Bromide.—It was not expected that this quaternary ammonium compound would enter the brain, and it was therefore not surprising to find that amounts as large as 10 mg./kg. did not impair the response to reticular stimulation. A transient slowing of the electrocortical potentials occasionally followed the injection and seemed to be associated with the brief hypotensive effect of the drug.

An intracerebroventricular cannula (Feldberg and Sherwood, 1953) was inserted in one experiment and 5 mg. methanthelinium was injected. No changes in the spontaneous electrical activity or in the response to reticular stimulation occurred during the ensuing hour.

It is thus conceivable that a drug such as methanthelinium might be valuable for premedicating animals used in this type of work when atropine or hyoscine is contraindicated because of the central effects described above.

Reversal of Hyoscine Effects by Physostigmine

In animals treated with hyoscine (as in Fig. 3*a*), it was found possible to reverse the effects of the drug on the spontaneous cortical potentials and on reticular activation of the electroencephalogram with physostigmine sulphate (0.2 mg./kg.) (Fig. 3*b*). This would appear to offer the most striking evidence for the existence of a cholinergic type of transmission somewhere within the reticular activating system.

Neostigmine does not appear to exert central effects (Bradley and Elkes, 1957), probably because of its quaternary nature.

Anti-nicotinic Agents

The results with hyoscine, which blocks reticular activation in very low doses, point strongly towards a cholinergic mechanism in which acetylcholine, or some other choline ester, is exerting a muscarine-like action. It was therefore considered important to test three drugs which dis-

play anti-nicotinic effects on the peripheral nervous system and can also enter the central nervous system to see whether they were capable of blocking reticular activation of the electroencephalogram.

Nicotine.—50 mg./kg. of nicotine base was administered in divided doses to each of two cats, to see whether the secondary paralysing actions on ganglia and skeletal muscle were also manifested on the reticular activating system. After the initial cardiovascular effects had subsided, the electroencephalographic voltage was somewhat lower and the frequency higher than before. Occasional convulsive spikes appeared. However, clear signs of further electroencephalographic activation were obtainable on reticular stimulation (Fig. 4*a*).

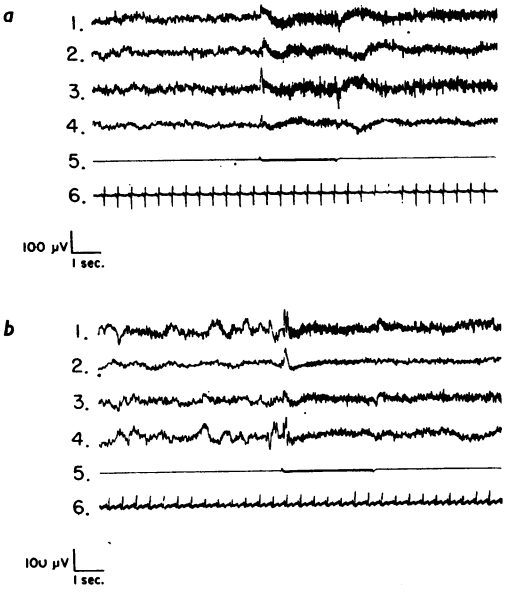

FIG. 4.—Electroencephalograms, channels as in Fig. 2. *a*, Low-voltage, moderately fast, spontaneous cortical potentials after intravenous nicotine base (50 mg./kg.). There is clear evidence of further activation on reticular stimulation. *b*, Failure of 10 mg./kg. mecamylamine hydrochloride to block reticular activation.

Mecamylamine Hydrochloride.—This potent ganglionic blocking agent, chemically a secondary amine, is known to enter the brain readily (Milne, Rowe, Somers, Muehrcke, and Crawford, 1957). Clinically, large doses may produce temporary psychoses or severe tremors (Harington and Kincaid-Smith, 1958).

We have given four cats intravenous injections of 10 mg./kg. mecamylamine and have been unable to demonstrate any change in the resting electroencephalographic pattern or in the activation produced by reticular stimulation (Fig. 4*b*), though ganglionic blockade was evident from a fall in blood pressure of about 30 mm. Hg.

Dihydro-β-erythroidine Hydrobromide.—This alkaloid has a well-known paralysing action on the skeletal neuromuscular junction which, in cats, is exerted in doses of about 0.5 mg./kg. It also blocks ganglionic transmission (Randall, 1951; Megirian, Leary, and Slater, 1955). Transmission in the spinal cord across the synapse between the motoneurone collateral and the Renshaw cell is readily blocked by intravenous injections of 0.1 mg./kg. (Eccles, Fatt, and Koketsu, 1954). Thus there is evidence that this alkaloid enters the central nervous system.

We have administered large intravenous doses of up to 20 mg./kg. of dihydro-β-erythroidine to three cats with no evidence of impairment of the response to reticular stimulation. The spontaneous electrocortical potentials were slowed only during the initial hypotensive response to the drug.

It is of interest to note that Starzl *et al.* (1951) used the parent alkaloid, β-erythroidine, for immobilizing animals during their experiments and did not mention any interference by the drug with electrocortical activity.

Thus we have no evidence from these experiments that the presumed cholinergic synapses in the ascending reticular system are influenced by drugs which display anti-nicotinic properties elsewhere in the nervous system.

Interneuronal Blocking Drugs

Previous investigators have shown that neither mephenesin (Domino, 1955; King, 1956) nor meprobamate (Gangloff, 1958) given in the usual doses depresses electrocortical activation following recticular stimulation. On the other hand barbiturates, which depress synapses in general, readily block such reticular activation (French *et al.*, 1953a).

Pentobarbitone Sodium.—Fig. 5*a* shows the typically slowed electrocortical potentials seen after 10 mg./kg. of pentobarbitone intravenously, illustrating failure of activation during or following reticular stimulation. In contradistinction to hyoscine, however, it was not possible to antagonize the blocking action of small doses of pentobarbitone by giving physostigmine. Nevertheless, 0.4 mg./kg. of the latter agent did increase the frequency of the spontaneous electrocortical potentials after pentobarbitone and caused a disappearance of sleep spindles (Fig. 5*b*).

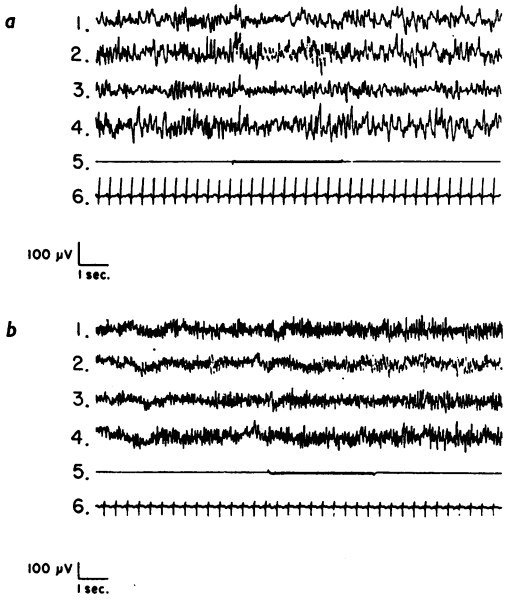

FIG. 5.—Electroencephalograms, channels as in Fig. 2. *a*, Typical failure of electrocortical activation after intravenous pentobarbitone (10 mg./kg.). *b*, Effect of 0·4 mg./kg. physostigmine on block of reticular activating system produced by pentobarbitone. Spontaneous electrical activity after pentobarbitone and physostigmine shows a greater component of fast activity than in *a*, but it will be seen that reticular stimulation in *b* produced no further activation.

Lignocaine Hydrochloride. — Bernhard and Bohm (1955) demonstrated some depression of spinal polysynaptic reflexes after intravenous lignocaine, and they suggested that the drug might also depress the bulbar reticular formation.

Fig. 6*a* shows a record taken from a cat which had been given 15 mg./kg. of lignocaine slowly enough to produce only a minor fall in blood pressure. The spontaneous potentials were immediately slowed and sleep spindles appeared. Electroencephalographic activation, however, readily occurred on reticular stimulation, though its duration following the stimulus was often shortened. Thus lignocaine, at this dose, appeared to be causing some depression of the reticular activating system. The effects of the drug persisted for only about 15 min.

It was found that the electroencephalographic effects of lignocaine, whilst they lasted, could be promptly abolished by giving physostigmine. This led us to consider the possibility of an atropine-like effect of lignocaine. We therefore tested the latter against the depressor responses to intravenous injections of 2 μg. of carbachol in a non-atropinized cat. Injections of 5 mg./kg. lignocaine intravenously abolished, for periods of about 1 min., the depressor effect of carbachol. The significance of this will be discussed later. It is of interest to note that Peterson (1955a) detected some anti-muscarinic activity in procaine.

Large injections of lignocaine (30 mg./kg.) resulted in severe hypotension and bradycardia, making it impossible to distinguish drug effects on the brain from ischaemic effects.

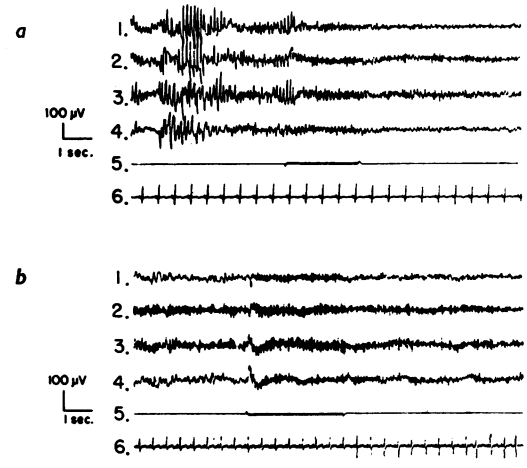

FIG. 6.—Electroencephalograms, channels as in Fig. 2. *a*, Cortical potentials following slow intravenous injection of lignocaine hydrochloride (15 mg./kg.). Slowed spontaneous activity with sleep spindles; electroencephalographic activation still evoked by reticular stimulation; duration of such activation usually reduced by lignocaine. *b*, Effect of dihydroergotamine methanesulphonate (1 mg./kg.). Spontaneous fast activity in channels 2 and 3, but clear signs of activation by reticular stimulation in channels 1 and 4.

Adrenergic Blocking Agents

The fact that adrenaline administered systemically can bring about electroencephalographic activation (Bonvallet *et al.*, 1954) has led many to suspect the existence of an adrenergic type of neurohumoral transmission within certain parts of the ascending reticular system. We have therefore tested some drugs which block peripheral adrenergic neuro-effector systems for possible actions on the reticular system.

Ergot Derivatives.—Neither ergotamine tartrate nor dihydroergotamine methanesulphonate, in amounts many times those producing full adrenaline-reversal, were capable of depressing reticular activation. The record of Fig. 6*b* was taken 10 min. after injecting 1 mg./kg. of the hydrogenated alkaloid; the spontaneous potentials tend to be fairly fast in frequency, but clear signs of further activation could be seen on reticular stimulation.

Phenoxybenzamine Hydrochloride.—Intravenous injections of this compound, given in divided amounts up to 10 mg./kg., resulted in slowing of the spontaneous cortical potentials, perhaps due to a concomitant fall in blood pressure to about 55 mm. Hg produced by the drug. Nevertheless, we were able to elicit clear-cut signs of reticular activation in the electroencephalogram.

Azapetine Phosphate.—This drug produced a smaller hypotensive reaction than did phenoxybenzamine and, in moderate doses, electroencephalographic activation was not prevented. Injections of 30 mg./kg. resulted in a convulsive type of electroencephalographic pattern from which it was not possible to discern the effects of reticular stimulation.

Choline 2 : 6-Xylyl Ether Bromide.—The adrenergic blockade produced by this drug (Hey and Willey, 1954) differs fundamentally from that of the previously mentioned compounds, since it reduces the amount of adrenergic transmitter released (Exley, 1957); this may be due to interference with the biosynthesis of the mediator (Exley, 1956; Coupland and Exley, 1957; Bain and Fielden, 1957).

Intravenous injections of 10 mg./kg. of the drug failed to modify either the spontaneous electroencephalogram or the response to reticular stimulation. Being a quaternary compound, it probably failed to enter the central nervous system. However, an injection of 2.5 mg. directly into the right cerebral ventricle lilkewise failed to produce any change.

The adrenergic blocking drugs as a whole therefore do not seem to exert any marked action on the reticular activating system.

Discussion

Evidence for the participation of a cholinergic mechanism at some point within the ascending reticular system is indirect, and has been well reviewed by Himwich and Rinaldi (1957). In particular, they have demonstrated electroencephalographic activation in rabbits following intracarotid injection of either acetylcholine or dyflos, and have further shown that atropine can both reverse these effects and block the activation following electrical stimulation of the reticular formation. Many of the antiparkinson drugs also block the effects of reticular stimulation (Rinaldi and Himwich, 1955c).

We were interested in trying to determine whether the action of the hypothetical cholinergic transmitter within the ascending reticular system was muscarine-like or nicotine-like. To some extent we have succeeded in the attempt, since we have found that the anti-nicotinic agents dihydro-β-erythroidine and mecamylamine, and large doses of nicotine itself, certainly fail to block reticular activation of the electroencephalogram. It is relevant that the nicotinic cholinergic type of synapse between the motorneurone collateral and the Renshaw cell of the cord can be readily blocked by dihydro-β-erythroidine (Eccles *et al.*, 1954). This synapse therefore seems to differ, at least pharmacologically, from the inferred cholinergic synapses associated with the reticular activating system.

On the other hand, it seems that the hypothetical cholinergic transmitter, occurring somewhere in the reticular activating system, displays properties analogous in some respects to the muscarinic actions of acetylcholine. We have confirmed the observation of Rinaldi and Himwich (1955a) that atropine depresses the reticular activating system. More recently, Kuehn and Schallek (1958) have reported that hyoscine is about one hundred times as potent as atropine in this respect. We have also found a high order of blocking activity in hyoscine, and have demonstrated a clear antagonism of this blockade by physostigmine. The marked difference in potency between atropine and hyoscine is, however, surprising and difficult to explain in the light of the moderate difference of about twofold in the peripheral anti-muscarinic activities of the two alkaloids. That hyoscine has marked central depressant effects, not seen clinically with atropine, is too well known for elaboration here; it is a salient example of the profound pharmacological change that can result from a slight alteration in chemical structure.

The site of the depressant action of hyoscine on the reticular activating system has not been established; Bradley and Key (1958) believe it to be at higher levels than the mid-brain reticular formation, possibly within the diffuse thalamic projection. If hyoscine were acting on the reticular formation itself, then the animal presumably would exhibit block of reticular activation of the electroencephalogram together with behavioural somnolence, as do cats with induced lesions of the rostral mid-brain reticular substance (Lindsley, Schreiner, Knowles, and Magoun, 1950). But Bradley and Elkes (1957) have clearly shown that hyoscine does not enforce behavioural sleep in cats.

It is not easy to explain the differences in the reactions of the various central and peripheral synapses to the interneuronal blocking drugs and

barbiturates. Reference has already been made to the failure of mephenesin or meprobamate to influence the reticular activating system, in spite of their depressant actions on the polysynaptic spinal pathways. Local anaesthetics, such as procaine (Peterson, 1955b) or lignocaine (Bernhard and Bohm, 1955), are capable of depressing the cord reflexes; in high concentrations they may also depress transmission in sympathetic ganglia. We have found that moderate doses of lignocaine invariably slow the spontaneous electrical activity, cause the appearance of sleep spindles and may reduce the duration of electroencephalographic activation following reticular stimulation. The fact that physostigmine can promptly reverse these effects suggests to us that the mode of action of lignocaine on the reticular activating system might resemble that of atropine more closely than that of pentobarbitone. Our demonstration of a very transitory anti-muscarinic effect of lignocaine on the cardiovascular system raises the possibility that the drug may fix more firmly to structures in the central nervous system, and hence produce atropine-like effects of longer duration than it does peripherally. If the central action is like that of atropine or hyoscine then lignocaine would not be expected to cause behavioural sleep in unrestrained animals.

The barbiturates may be considered to show pharmacological actions on the nervous system which to some extent overlap those of the anti-muscarinic, anti-nicotinic, and interneuronal blocking agents. Barbiturates depress reticular activation, ganglionic transmission, and the cord reflexes, but they do not have peripheral atropine-like effects (Exley, 1954) or skeletal neuromuscular-blocking effects (Quilliam, 1955). Our observation that depression of reticular activation by pentobarbitone cannot be overcome with physostigmine is in keeping with the fact that barbiturate narcosis cannot be antagonized with physostigmine in the unrestrained cat (Bradley and Elkes, 1957).

In conclusion, it would seem that present evidence for a cholinergic type of interneuronal transmission within the reticular activating system is more abundant than that for an adrenergic process. Admittedly, catechol amines are found in brain tissue, and injected adrenaline may under certain circumstances activate the electroencephalogram, but these facts can only provide suggestive evidence of an extremely indirect nature. There are some facts which are in direct opposition to such an adrenergic transmission concept. First, reserpine depletes brain tissue of its noradrenaline content (Brodie, Olin, Kuntzman, and Shore, 1957), but moderate to high doses of reserpine do not appear to block activation of the electroencephalogram by reticular stimulation (Rinaldi and Himwich, 1955d; Killam and Killam, 1956); second, Kuehn and Schallek (1958) have been unable to demonstrate any change in stimulus threshold for reticular activation after treatment of cats with the amine oxidase inhibitor iproniazid; and, third, we have been unable to detect depressant actions on the reticular activating system in five different adrenergic blocking drugs.

Further elucidation of the problems of central synaptic transmission still awaits unequivocal experiments demonstrating the liberation of specific transmitters following controlled central nervous activity. Meanwhile, however, neuropharmacological approaches can offer much in the way of logical elimination.

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