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DIFFERENTIAL ACTIONS OF *m* AND *n* CHOLINERGIC AGONISTS ON THE BRAINSTEM ACTIVATING SYSTEM*

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Summary—The differential actions of i.v. arecoline and nicotine were determined on neocortical and limbic system EEG activation in acute rostral and caudal midbrain transected cats. All animals were prepared under diethyl ether anesthesia and after surgery, paralyzed with decamethonium and maintained on artificial respiration. The peripheral effects of these cholinergic agonists were reduced by methyl atropine (250 μg/kg) and/or trimethidinium (1 mg/kg) pretreatment.

In the caudal midbrain transected preparation, nicotine (20–40 μg/kg) induced marked EEG activation in both the neocortex and hippocampus. After bilateral lesions of the midbrain reticular formation in the same preparation, EEG activation was not observed with nicotine in doses up to 100 μg/kg. The EEG effects of nicotine were blocked by atropine (1 mg/kg) and mecamylamine (1 mg/kg) but not trimethidinium (1 mg/kg). In the rostral midbrain transected preparation no EEG activation was noted with nicotine in doses up to 100 μg/kg. Sporadic sharp waves appeared in the hippocampus with the larger doses indicating a convulsant site of action above the level of transection.

Arecoline induced dissociation of the EEG in the hippocampus and neocortex in doses of 20–40 μg/kg in the rostral midbrain transected cat. Marked hippocampal slow "arousal" waves with no desynchronization of the neocortical EEG were seen. These effects of arecoline were blocked by atropine. In the caudal midbrain preparation, even after bilateral lesions of the midbrain reticular formation which blocked nicotine activation, arecoline (20–40 μg/kg) still induced hippocampal slow 'arousal' waves without neocortical desynchronization. With doses of 100 μg/kg of arecoline both neocortical and hippocampal EEG activation was noted.

It is concluded that the site of nicotine on the rostral forebrain activating system is located primarily in the midbrain reticular formation, whereas arecoline acts on the midbrain reticular formation as well as above the level of the mesencephalon.

RINALDI and HIMWICH (1955a, b) and BRADLEY and ELKES (1957) were among the first to provide evidence for a cholinergic mechanism in mesen-diencephalic structures mediating EEG activation. In the intervening years many other investigators have elaborated this concept (KILLAM, 1962; SILVETTE *et al.*, 1962; WHITE and BOYAJY, 1959; WHITE, 1963; LONGO, 1966; STÜMPF, 1965; VOTAVA, 1967). It has been shown that muscarinic (*m*) and nicotinic (*n*) cholinergic components are involved in EEG activation as evidenced by the use of selective *m* and *n* cholinergic agonists and antagonists (ILYUTCHENOK, 1962; DOMINO *et al.*, 1967; YAMAMOTO and DOMINO, 1967). Furthermore, recent neurophysiological advances have shown that the mesencephalic reticular formation affects predominantly the neocortex while the hypothalamus affects predominantly the limbic system. For example, the presence of a specific activating mechanism in the hypothalamus on the hippocampal EEG has been shown using the rostral midbrain transected (RMT) cat, which lacks the

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midbrain reticular formation (KAWAMURA *et al.*, 1961; KAWAMURA and OSHIMA, 1962; KAWAMURA and DOMINO, 1968). In the RMT cat, threshold electrical stimulation of the hypothalamus induced hippocampal EEG activation without desynchronization of the neocortex. Although the midbrain reticular formation and hypothalamus have a close relationship, especially in the intact animal, there is a functional differentiation. In other words, the hippocampal EEG reflects primarily hypothalamic activation, whereas neocortical desynchronization reflects primarily midbrain reticular influences.

In view of the fact that the neocortical and limbic activating mechanisms can be dissociated, it was important to determine if this would apply to *m* and *n* cholinergic agonists which produce EEG activation. This paper describes the differential activating effects of arecoline and nicotine, typical *m* and *n* cholinergic agonists which readily penetrate the blood–brain barrier.

METHODS

Thirty cats of both sexes, weighing 2·3–4·0 kg were used. In eighteen cats, the rostral midbrain was transected at the junction between the diencephalon and midbrain. In twelve cats, the caudal midbrain was transected from the colliculi down to the anterior border of the pons. Tracheotomy was performed under diethyl ether anesthesia and the cat mounted in a stereotaxic apparatus. The forearm veins were cannulated for drug injection. The wound margins were infiltrated with 1% xylocaine. The method of transecting the brainstem at the junction of diencephalon and midbrain (RMT) has been described elsewhere (KAWAMURA and DOMINO, 1968). For the caudal midbrain transection (CMT), a trephine hole was made in the skull overlying the cerebellum and was made posterior to the bony tentorium. Then the cerebellum was removed by aspiration and the fourth ventricle exposed. The brainstem was transected just behind the bony tentorium using a small curved spatula. Ether was discontinued after surgery and decamethonium was injected i.v. Respiration was maintained by an artificial respirator (Harvard Pump). Blood pressure was recorded from the femoral artery with a Statham transducer connected to one channel of a Grass polygraph. The neocortical EEG was recorded using phonograph needle electrodes placed on the skull such that the tips rested on the dura overlying the frontal and parietal cortices. In several cases, the frontal bone was used as reference. For recording electrical activity from the hippocampus and for stimulating the brainstem, concentric needle electrodes insulated except at the tips were inserted stereotaxically according to the atlas of JASPER and AJMONE-MARSAN (1954). Body temperature was monitored during the experiment with a rectal thermometer and maintained at 37–38°C with a heating pad placed under the animal.

For drug injection, freshly prepared nicotine tartarate (40 μg/ml), arecoline hydrochloride (40 μg/ml), atropine methyl nitrate (250 μg/ml) and trimethidinium (1 mg/ml) were used. All drugs were diluted with physiological saline and given as base. Injections were given over 30 sec. After each injection, the cannula was flushed immediately with 2 ml of physiological saline.

Electrical activity was recorded by means of a Grass Model 7 polygraph (time constant: 0·1 sec.) Responsiveness of the forebrain preparation was checked by high frequency electrical stimulation of the hypothalamus with square wave pulses (100 cycles/sec, 1 msec, 2–5 V for 5 sec) delivered from a Grass S 8 stimulator. At the end of an experiment, the brain was perfused with 10% formalin. Completeness of transection and electrode locations were verified histologically, using cresyl violet or thionine stained serial sections.

RESULTS

Effects of cholinergic agonists on the RMT forebrain

The i.v. injection of small amounts of arecoline induced marked dissociation of the EEG between the neocortex and the hippocampus. The threshold dose inducing hippocampal slow waves as an indication of activation was usually 10 μg/kg. In several preparations, however, 5 μg/kg induced activation. A larger dose of 20 μg/kg, induced very marked dissociation between the hippocampal and neocortical EEG. After 20 μg/kg of arecoline i.v. and upon recovery from the fall in arterial blood pressure, the hippocampal EEG changed from irregular fast waves with spikes to a regular slow 3 cycles/sec activation pattern, while the neocortical EEG showed no apparent change except for slight enhancement of spindle bursts (see Fig. 1). The hippocampal regular slow waves continued for 3–10 min with a gradual return to the pre-drug state. To prove that this EEG change was not due to the fall in systemic blood pressure, methyl atropine (250 μg/kg, i.v.) was given to reduce the peripheral effects of arecoline. In the same cat, half an hour after pretreatment with methyl atropine, the same dose of arecoline no longer altered blood pressure (Fig. 1,

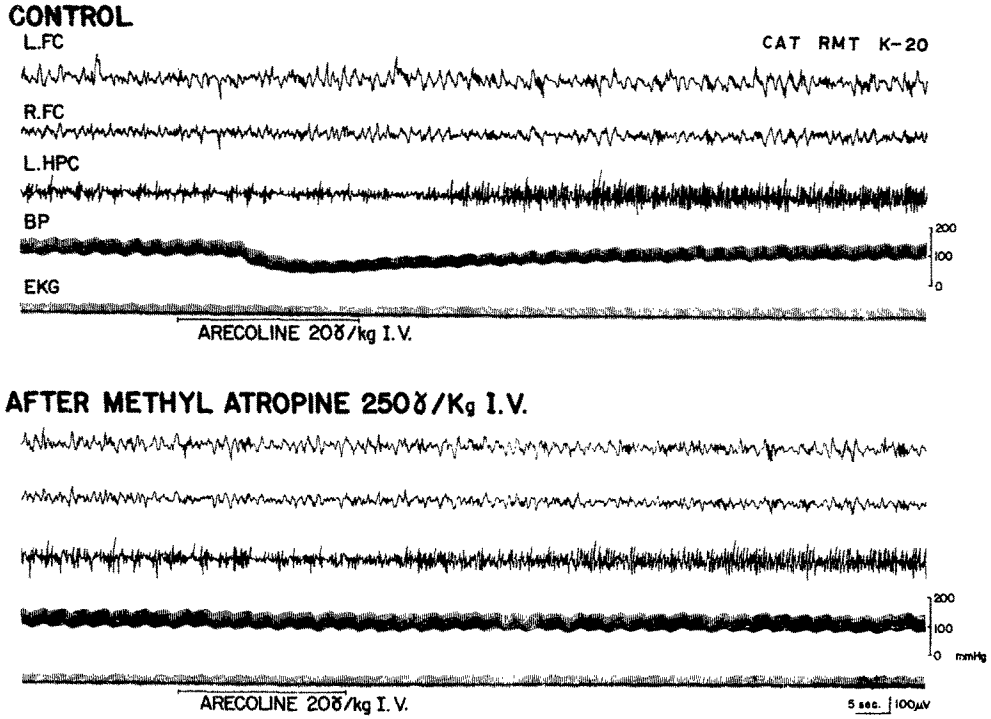

FIG. 1. Hippocampal EEG activation induced by arecoline in the rostral midbrain transected cat. Upper panel: control. An injection of 20 μg/kg of arecoline i.v. induced hypotension with 3 cycles/sec regular slow wave activation of the hippocampus. Note that at this dosage the drug did not induce neocortical EEG desynchronization. Lower panel: after methyl atropine pretreatment. Although arecoline-induced hypotension was blocked, marked hippocampal activation was still seen, but at a frequency of 2·5 cycles/sec. The abbreviations in this and following illustrations are: L. FC, left frontal cortex–frontal bone; R. FC, right frontal cortex–frontal bone; L. FC-PC, left frontal cortex–parietal cortex; R. FC-PC, right frontal cortex–parietal cortex; L. HPC, left hippocampus; monopolar recordings were taken to the nasal bone B.P., blood pressure; EKG, electrocardiogram, lead 1.

lower panel). Hippocampal activation was elicited with the same time course as previously, although the frequency of the hippocampal regular slow waves was slightly lower (2·0–2·5 cycles/sec).

As the dose of arecoline was increased, the neocortical EEG was more affected. In responsive RMT preparations whose brainstem was transected at the junction of midbrain and diencephalon, increasing the dose of arecoline to 50–100 μg/kg produced suppression of slow waves and spindle bursts in the neocortex in addition to continued marked hippocampal activation. Low voltage fast waves appeared in the neocortex with doses of 100 μg/kg or more of arecoline. Desynchronization was observed more readily when the transection was caudal and part of the midbrain remained with the forebrain. EEG changes bore no relation to blood pressure. The EEG differences between the effects of a small (20 μg/kg) and large (100 μg/kg) doses of arecoline are illustrated in Fig. 2. In the RMT preparation,

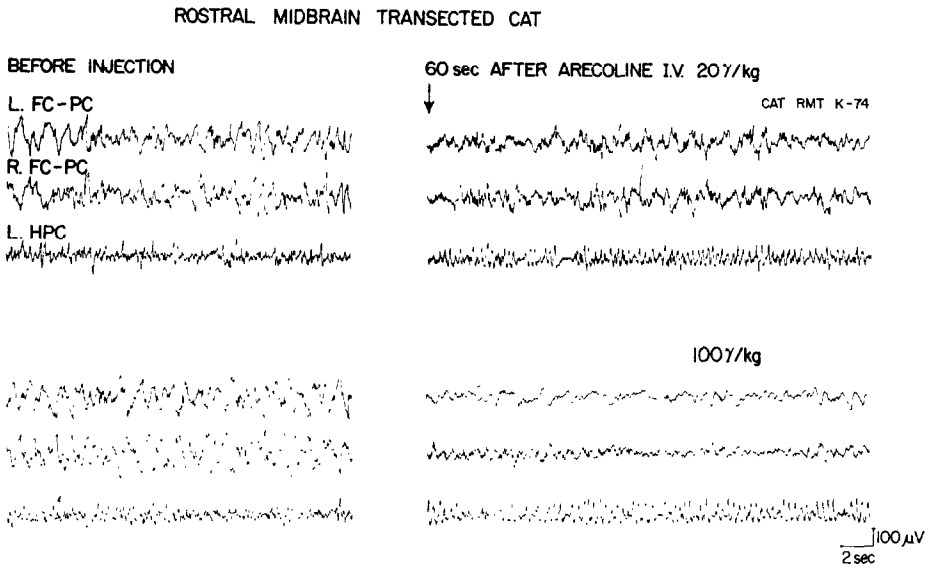

FIG. 2. Effect of increasing doses of arecoline on the EEG of the rostral midbrain transected cat. After transection the animal was given 250 μg/kg of methyl atropine and tested within half an hr. Upper panel: before arecoline injection the neocortical and hippocampal EEG show the typical slow waves and spikes. About 60 sec after 20 μg/kg of arecoline i.v. hippocampal regular slow waves were observed while the neocortical EEG showed no desynchronization but some increase in fast waves and a slight suppression of slow wave activity. Lower panel: before injection typical slow wave and spike-like activity was present. After 100 μg/kg of arecoline, there was a suppression of slow waves and appearance of a low voltage fast wave pattern in the neocortex as well as hippocampal regular slow wave activation.

pretreated with 250 μg/kg methyl atropine half an hour previously, 20 μg/kg of arecoline produced marked hippocampal activation, while the neocortex showed only an increase in fast activity superimposed on a background of slow waves. However, following 100 μg/kg of arecoline the neocortex showed marked suppression of slow waves and a low voltage fast pattern appeared. Thus, in the RMT preparation, small doses of arecoline induced marked dissociation of the EEG of the neocortex and limbic system. With large doses, the neocortex showed desynchronization. Atropine (1 mg/kg, i.v.) blocked the EEG activating effect of 20–100 μg/kg of arecoline in the RMT forebrain.

In the RMT preparation, 20–40 μg/kg of nicotine did not produce significant activation in the hippocampus (Fig. 3) although similar doses caused marked activation of both neocortex and hippocampus in the intact or CMT cat. In the RMT preparation without trimethidinium pretreatment, 20 μg/kg of nicotine induced a marked hypertension associated with sporadic sharp waves localized to the hippocampus. After 1 mg/kg of trimethidinium the pressor response to nicotine was completely blocked and no EEG change was seen in the hippocampus. With larger doses of 200–500 μg/kg, hippocampal sharp waves were some-

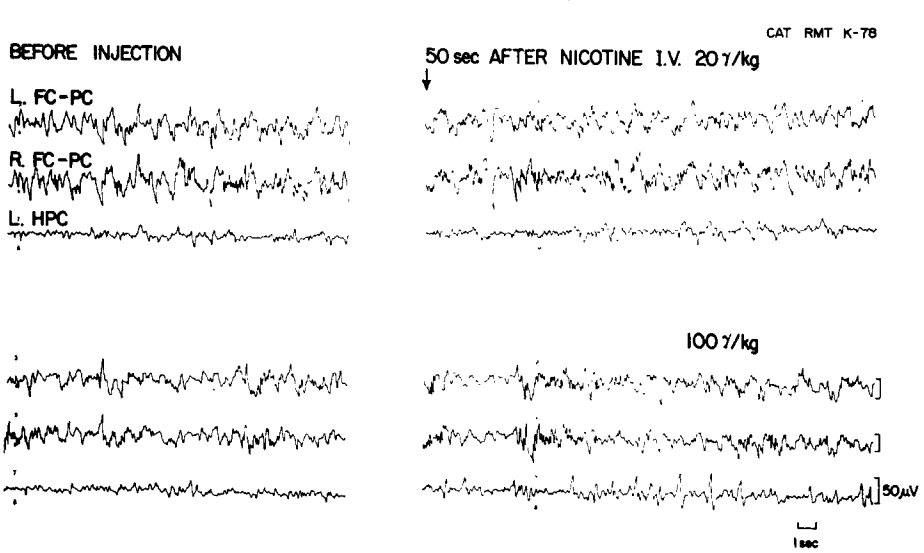

FIG. 3. Effect of increasing doses of nicotine on the EEG of the rostral midbrain transected cat. Following transection 1 mg/kg, i.v. of trimethidinium was given as pre-treatment. Upper panel; 1 hr later 20 μg/kg of nicotine i.v. had no effect in contrast to arecoline (see Fig. 2). Lower panel: A dose of nicotine 5 times as great (100 μg/kg) induced sporadic sharp waves in the hippocampus although there was no significant change in the neocortical EEG slow wave pattern.

times followed by seizure discharges. In several cats, enhancement of EEG activation by nicotine (20–40 μg/kg) was seen when nicotine was injected after arecoline (20–40 μg/kg) in both RMT and CMT preparations. On the other hand, when arecoline was given after nicotine there was no evidence of any influence from the nicotine. Therefore, in order to draw conclusions concerning the actions of nicotine, arecoline was never given prior to nicotine in these experiments.

Effects of nicotine and arecoline in the CMT forebrain

If the midbrain was transected caudally at the junction of the pons, both nicotine and arecoline induced neocortical desynchronization as well as hippocampal regular slow "arousal" waves. Such EEG activation was induced even when blood pressure remained constant by pretreatment with trimethidinium for nicotine and methyl atropine for arecoline. Whenever a blood pressure change occurred, the EEG changes were of longer duration and consisted of waves of higher frequency. After bilateral lesions of the midbrain tegmentum, the EEG activating effect of nicotine was completely blocked. Figure 4 illustrates

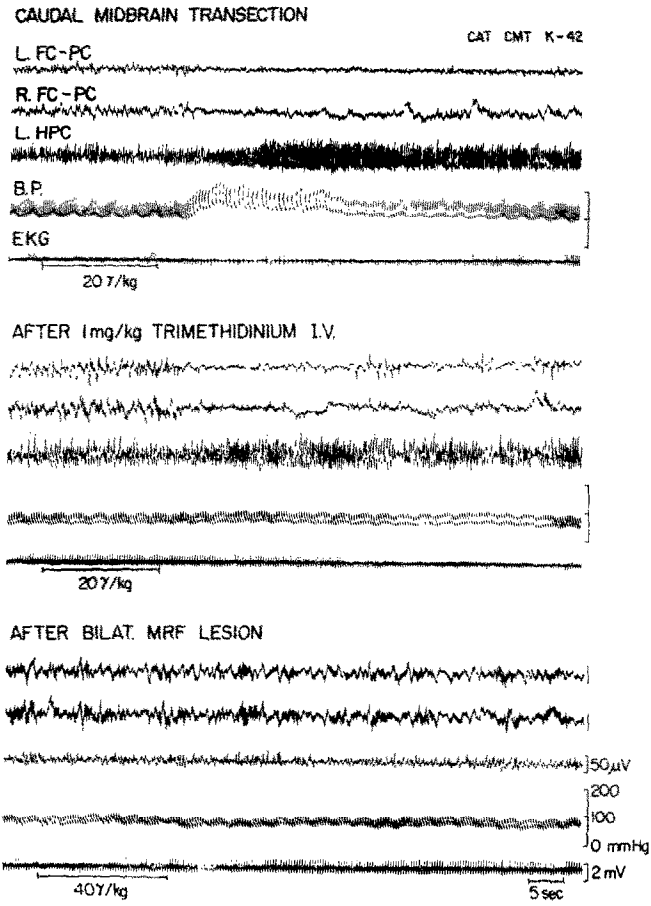

FIG. 4. EEG activation induced by nicotine in the caudal midbrain transected cat. Upper panel: the i.v. injection of 20 μg/kg of nicotine produced marked hypertension and dramatic EEG activation both in the neocortex and hippocampus. Middle panel: 1 hr after 1 mg/kg of trimethidinium i.v. the same dose of nicotine still produced EEG activation in the absence of a change in blood pressure. The intensity of EEG activation was not as great, although still clearly evident. Lower panel: after bilateral lesions of the midbrain reticular formation, even a larger dose of nicotine (40 μg/kg) did not activate the EEG. This was true even though the interval between nicotine injections was more than 1½ hr to avoid tachyphylaxis.

this phenomenon in a preparation in which the caudal midbrain was transected. To further eliminate afferent impulses from the periphery which might cause activation, the optic nerves and olfactory tracts were cut bilaterally in three cats. Similar results were obtained indicating that these afferents have no effect on forebrain activation induced by nicotine or arecoline.

In intact animals nicotine in doses of 20 μg/kg, i.v. induced marked hypertension (up to 200 mm Hg) with bradycardia, simultaneous desynchronization of the neocortical EEG and enhancement of hippocampal regular slow "arousal" waves as illustrated in the recording in the upper panel of Fig. 4. After pretreatment with 1 mg/kg of trimethidinium 1 hr previously a second dose of nicotine (20 μg/kg, i.v.) produced no change in blood pressure but neocortical desynchronization and hippocampal "arousal" waves still appeared.

However, the duration of activation and the frequency of the EEG waves were lower than before trimethidinium. Bilateral electrolytic lesions of the midbrain reticular formation in such preparations completely prevented the EEG actions of nicotine even in doses of 40 μg/kg, i.v. The location of the midbrain tegmental lesions in one typical cat in which an extensive bilateral lesion prevented nicotine induced EEG activation is illustrated diagrammatically in Fig. 5. After such midbrain reticular formation lesions, nicotine no longer

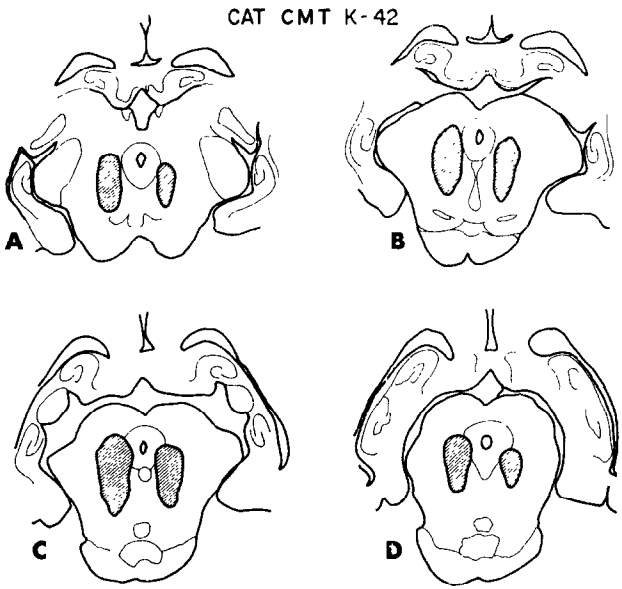

FIG. 5. Schematic diagram of the localization of the lesions of the midbrain reticular formation which blocked nicotine induced activation. The areas outlined by the slanted lines indicate the size of the lesion which occupies the area from the tip of the midbrain to the caudal portion of the midbrain near the pons.

activated the neocortical EEG, even in doses up to 100 μg/kg. However, arecoline (20–40 μg/kg) still induced marked activation in the hippocampus, and both neocortical and hippocampal activation with larger doses. These effects are illustrated in Fig. 6 in the EEG recordings selected from one cat. Trimethidinium (1 mg/kg, i.v.) was given before nicotine and methyl atropine (250 μg/kg) before arecoline. In the CMT preparation, a small amount of nicotine (20 μg/kg, i.v.) induced EEG activation in both the neocortex and hippocampus. After bilateral lesions of the midbrain reticular formation, doses up to 100 μg/kg showed no effect on the EEG. In such a preparation following arecoline (40 μg/kg, i.v.) the hippocampal EEG showed a clear change from the preinjection pattern of irregular fast waves with spikes to a regular slow "arousal" pattern, but the neocortex showed little desynchronization. With this dose of arecoline there was an obvious dissociation between the neocortical and hippocampal EEG activation in the RMT preparation. These effects were blocked by atropine (1 mg/kg, i.v.) In addition, in the CMT preparation, atropine blocked both arecoline and nicotine EEG activation.

In an additional series of experiments, electrolytic lesions were made in the interpeduncular nucleus in 5 CMT cats. In these lesioned preparations nicotine activation of the

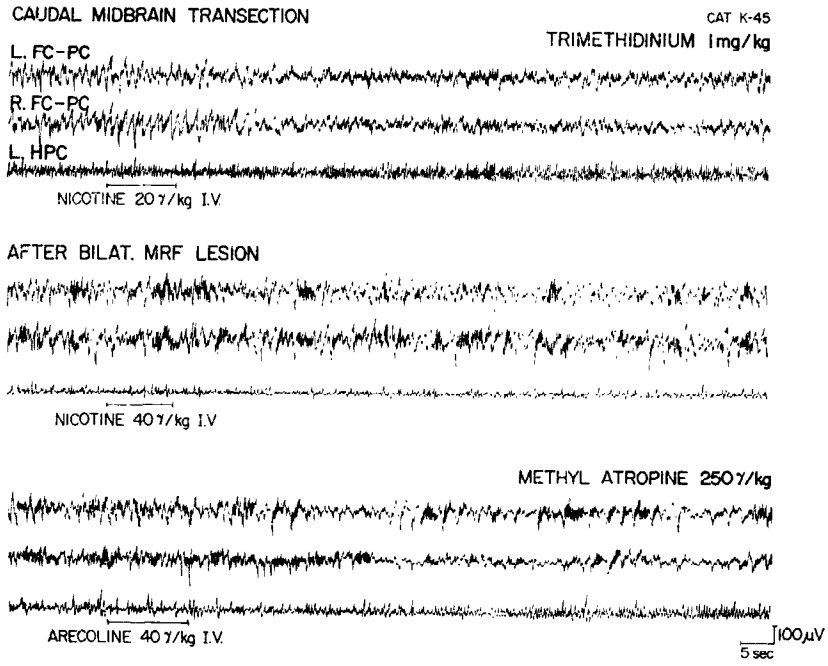

FIG. 6. Differential effects of nicotine and arecoline in a caudal midbrain transected cat with bilateral lesions of the reticular formation. Upper panel: following trimethidinium pretreatment nicotine (20 μg/kg) induced desynchronization of the neocortex and hippocampal slow waves for a short period of time. Middle panel: after bilateral lesions of the midbrain reticular formation injection of nicotine in an even larger dose (40 μg/kg) did not produce EEG activation. Lower panel: in contrast to the lack of effects of nicotine, after methyl atropine pretreatment (250 μg/kg) a similar dose of arecoline caused the appearance of hippocampal slow wave activation in the absence of neocortical EEG desynchronization.

neocortical and hippocampal EEG was still observed indicating that the interpeduncular nucleus is not critical for this phenomenon.

DISCUSSION

The midbrain reticular formation is an indispensable structure for the activation of the forebrain EEG by nicotine. A lesion in this area prevents nicotine induced tonic activation of the neocortical EEG. These conclusions are based upon experiments in the CMT preparation in which most afferent impulses from the periphery were blocked as well as in additional preparations in which the optic nerves and olfactory tracts were transected. The fact that a small amount of arecoline can activate the hippocampal EEG in a deafferentiated forebrain, which lacks the midbrain reticular formation bilaterally, indicates that arecoline has a direct effect on a forebrain activating mechanism, possibly on the hypothalamus as based upon the results from our earlier stimulation experiments in RMT cats (KAWAMURA and DOMINO, 1968).

The EEG activating effects of arecoline or nicotine in the brainstem transected forebrain is not due to the action of the drug on the transected surface where the blood–brain barrier is seriously damaged, because both drugs are known to pass the blood–brain barrier readily and the xylocaine infiltration of the surface of the transected brainstem has no influence on their activating effect.

Nicotine and arecoline have marked peripheral cardiovascular effects. In order to block the direct effect of systemic blood pressure changes on the mesen-diencephalic pressure sensitive areas (BAUST *et al.*, 1963), appropriate doses of cholinergic antagonists for each drug were given as pretreatment. Trimethidinium, 1 mg/kg, i.v. was given to reduce the peripheral effects of nicotine and methyl atropine, 250 μg/kg, i.v. to reduce the peripheral effects of arecoline. Such pretreatment successfully prevented any marked changes in systemic blood pressure. Whereas arecoline induced marked hippocampal EEG changes in the RMT preparation, nicotine did not. The lack of effect of nicotine on hippocampal activation even though there was marked hypertension (prior to trimethidinium) indicated further that the EEG effects observed were basically unrelated to changes in blood pressure. In addition, the i.v. injection of large doses of epinephrine (5 μg/kg) induced a marked increase in blood pressure without EEG activation in the RMT preparation, indicating that hypertension was not the main factor responsible for EEG activation. Thus, one can conclude that the cholinergic agonists themselves are responsible for inducing EEG activation, although their peripheral cardiovascular effects do prolong and enhance their action on the brainstem activating system.

The EEG desynchronizing actions of nicotine have been reported in the inferior collicular-midpontine transected rabbit, cat and dog (KNAPP and DOMINO, 1962), as well as in the *cerveau isolé* rabbit (FLORIS *et al.*, 1963). It should be noted that in all these preparations, the midbrain reticular formation was partically connected to the forebrain. Similarly, in the present series of experiments, the more caudal the midbrain transection, the easier it was to activate the EEG with nicotine. It would appear that the midbrain reticular formation is the most important structure for EEG activation of the forebrain by nicotine. The more caudally located reticular formation appears to enhance this effect. This finding is in accord with the results of earlier investigators. Even 100 μg/kg of nicotine could not induce significant neocortical or hippocampal EEG activation in the RMT preparation which lacks the midbrain reticular formation. However, arecoline in small doses in the same RMT preparation easily induced hippocampal EEG activation. Likewise, in the CMT preparation in which the midbrain reticular formation was destroyed bilaterally, nicotine did not activate the neocortical or hippocampal EEG, while arecoline still induced hippocampal activation.

Large doses of nicotine (1–5 mg/kg) are known to induce seizure discharges, particularly in the hippocampus (STÜMPF *et al.*, 1962; STÜMPF and GOGOLAK, 1967). According to SCHMITERLÖW *et al.* (1967), injection of ^{14}C-labeled nicotine localizes in high concentrations in the archicortex, especially in the molecular and pyramidal cell layers of the hippocampus. On the basis of the present experiments nicotine may produce hippocampal seizure discharges as a direct effect. It has also been shown by SCHMITERLÖW *et al.* (1967) that the interpeduncular nucleus also contains very high concentrations of injected ^{14}C-nicotine in the cat. However, a lesion of the interpeduncular nucleus in the CMT cat failed to block EEG activation induced by nicotine. Although the concentration of ^{14}C-nicotine in the midbrain reticular formation is not high (SCHMITERLÖW *et al.*, 1967) it seems that this area is either very sensitive to nicotine or part of a critical circuit of neurons responsible for inducing EEG activation. It appears that in small doses nicotine first activates the EEG as a result of stimulating the midbrain reticular formation, and in larger doses, induces seizures, initially in the hippocampus. Sporadic high frequency sharp waves, which were sometimes observed in the hippocampus in the RMT preparation following large doses of nicotine may have been the first sign of seizure activity. The failure of nicotine to induce hippocampal slow wave

activation in the RMT preparation indicates this action is indirect and involving the reticular formation below the level of transection.

It is of interest that there are relatively few cholinergic neurons in the brain that respond to nicotine. It has been shown by ECCLES *et al.* (1954, 1956) that the Renshaw cells in the spinal cord are stimulated by nicotine. The fact that mecamylamine blocks this action suggests that this is an *n* cholinergic action (UEKI *et al.*, 1961), although both *m* and *n* cholinergic receptors are present on Renshaw cells (CURTIS and RYALL, 1964). It is of interest that no *n* cholinergic neurons have been found in the neocortex (KRNJEVIĆ and PHILLIS, 1963a, b). However, BRADLEY and WOLSTENCROFT (1967) have reported that iontophoretic injection of nicotine in the brainstem activates some neurons. Although there are both nicotinic and muscarinic neurons in the brainstem the muscarinic cells have a much wider distribution. The present experiments suggest that the brainstem activating system contains *m* cholinergic neurons in both the midbrain and diencephalon while *n* cholinergic neurons are located primarily in the midbrain reticular formation. However, to prove this hypothesis, precise microphysiological studies clarifying such a cell distribution are necessary.

REFERENCES

BAUST, W., NIEMCZYK, H. and VIETH, J. (1963). The action of blood pressure on the ascending reticular activating system with special reference to adrenaline induced EEG arousal. *Electroenceph. clin. Neurophysiol.* **15:** 63–72,

BRADLEY, P. B. and ELKES, J. (1957). The effects of some drugs on the electrical activity of the brain. *Brain* **80:** 77–117.

BRADLEY, P. B. and WOLSTENCROFT, J. H. (1967). Effects of acetylcholine, nicotine and muscarine on brain stem neurons. *Ann. N.Y. Acad. Sci.* **142:** 15–20.

CURTIS, D. R. and RYALL, R. W. (1964). Nicotinic and muscarinic receptors of Renshaw cells. *Nature, Lond.* **203:** 652–653.

DOMINO, E. F., DREN, A. T. and YAMAMOTO, K. I. (1967). Pharmacologic evidence for cholinergic mechanisms in neocortical and limbic activating systems. *Progress in Brain Research*, Vol. 27, pp. 337–364. Elsevier, Amsterdam.

ECCLES, J. C., ECCLES, R. M. and FATT, P. (1956). Pharmacological investigations on a central synapses operated by acetylcholine. *J. Physiol.* **131:** 154–169, 1956.

ECCLES, J. C., FATT, P. and KOKETSU, K. (1954). Cholinergic and inhibitory synapses in a pathway from motor axon collaterals to motoneurons. *J. Physiol.* **216:** 524–562.

FLORIS, V., MOROCUTTI, G. and AYALA, G. F. (1963). Azione della nicotina sulla attivitá bioelectrica della corteccia del talamo e dell'ippocampo nel cognilio. Sull'azione di "arousal" e convulsivante primitiva sulle strutture ippocampo-talamiche. *Boll. Soc. ital. Biol. sper.* **38:** 407–410.

ILYUTCHENOK, R. I. (1962). The role of cholinergic systems of the brainstem reticular formation in the mechanism of central effects of anticholinesterase and cholinolytic drugs. *Pharmacological Analysis of Central Nervous Actions* (PATON, W. D. M. and LINGREN, P., Eds.), pp. 211–216. Macmillan, New York.

JASPER, H. H. and AJMONE-MARSAN, C. (1954). *A Stereotaxic Atlas of the Diencephalon of the Cat.* National Research Council of Canada, Ottawa.

KAWAMURA, H. and DOMINO, E. F. (1968). Hippocampal slow ("arousal") wave activation in the rostral midbrain transected cat. *Electroenceph. clin. Neurophysiol.* **25:** 471–480.

KAWAMURA, H., NAKAMURA, Y. and TOKIZANE, T. (1961). Effects of acute brainstem lesions on the electrical activity of the neocortex and the limbic system. *Jap. J. Physiol.* **11:** 564–575.

KAWAMURA, H. and OSHIMA, K. (1962). Effect of adrenaline on the hypothalamic activating system. *Jap. J. Physiol.* **12:** 225–233.

KILLAM, E. K. (1962). Drug action on the brainstem reticular formation. *Pharmac. Rev.* **14:** 175–223.

KNAPP, D. E. and DOMINO, E. F. (1962). Action of nicotine on the ascending reticular activating system. *Int. J. Neuropharmac.* **1:** 333–351,

KRNJEVIĆ, K. and PHILLIS, J. W. (1963a). Acetylcholine sensitive cells in the cerebral cortex. *J. Physiol* **166:** 296–327,

KRNJEVIĆ, K. and PHILLIS, J. W. (1963b). Pharmacological properties of acetylcholine-sensitive cells in the cerebral cortex. *J. Physiol.* **166:** 328–350.

LONGO, V. G. (1966). Behavioral and electroencephalographic effects of atropine and related compounds. *Pharmac. Rev.* **18:** 965–996.

RINALDI, F. and HIMWICH, H. E. (1955a). Alerting responses in actions of atropine in cholinergic drugs. *Archs Neurol. Psychiat. Chicago* **73:** 387–395.

RINALDI, F. and HIMWICH, H. E. (1955b). Cholinergic mechanism involved in function of mesodiencephalic activating system. *Archs Neurol. Psychiat. Chicago* **73:** 396–402.

SCHMITERLÖW, C. G., HANSSON, E., ANDERSSON, G., APPELGREN, L. E. and HOFFMAN, P. C. (1967). Distribution of nicotine in the central nervous systems. *Ann. N. Y. Acad. Sci.* **142:** 2–14.

SILVETTE, H., HOFF, E. C., LARSON, P. S. and HAAG, H. B. (1962). The actions of nicotine on central nervous system functions. *Pharmac. Rev.* **14:** 137–173.

STÜMPF, C. and GOGOLAK, G. (1967). Actions of nicotine upon the limbic system. *Ann. N.Y. Acad. Sci.* **142:** 143–158.

STÜMPF, C., PETSCHE, H. and GOGOLAK, G. (1962). The significance of the rabbit's septum as a relay station between the midbrain and the hippocampus—II. The differential influence of drugs upon both the septal cell firing pattern and the hippocampus theta activity. *Electroenceph. clin. Neurophysiol.* **14:** 212–219.

UEKI, S., KOKETSU, K. and DOMINO, E. F. (1961). Effects of mecamylamine on the Golgi recurrent collateral-Renshaw cell synapse in the spinal cord. *Exp. Neurol.* **3:** 141–148.

VOTAVA, Z. (1967). Pharmacology of the central cholinergic synapses. *Ann. Rev. Pharmac.* **7:** 223–240.

WHITE, R. P. (1963). Relationship between cholinergic drugs and EEG activation. *Archs int. Pharmacodyn. Thér.* **145:** 1–17.

WHITE, R. P. and BOYAJY, L. D. (1959). Comparison of physostigmine and amphetamine in antagonizing the EEG effects of CNS depressants. *Proc. Soc. exp. Biol.* **102:** 479–483.

YAMAMOTO, K. I. and DOMINO, E. F. (1967). Cholinergic agonist-antagonist interactions on neocortical and limbic EEG activation. *Int. J. Neuropharmac.* **6:** 357–373.