Pharmacology Biochemistry and Behavior, Vol. 2, pp. 531–536. ANKHO International Inc., 1974. Printed in the U.S.A.

EEG and Heart Rate Changes Elicited by Chemical Stimulation of the Lateral Hypothalamus[1]

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(Received 15 November 1973)

CARMONA, A. AND J. L. SLANGEN. *EEG and heart rate changes elicited by chemical stimulation of the lateral hypothalamus.* PHARMAC. BIOCHEM. BEHAV. 2(4) 531–536, 1974. – Male rats with a permanently implanted cannula in the feeding area of the hypothalamus were tested for a reliable eating response to adrenergic and a reliable drinking response to cholinergic stimulation. Later on the rats were curarized and assigned at random to one of two groups in which the effects of the intrahypothalamic administration of drugs upon EEG cortical activity and upon cardiac activity were investigated. A reliable increase in the voltage of the EEGs and a decrease in the EEG frequency were elicited by injecting 1 μl of norepinephrine (40×10^{-9} moles). Increase in the EEG frequency and no change in the amplitude of the EEG's were observed after the injection of 1 μl of carbachol (2.4×10^{-9} moles). Both drugs decreased cardiac frequency but larger effects were elicited by carbachol. The results support the hypothesis that carbachol activated a complex parasympathetic centre while norepinephrine activated an hypothalamic sympathetic-inhibitory pathway.

Rat Intrahypothalamic injection Chemical stimulation Norepinephrine Carbachol EEG recording EEG counting device d-Tubocurarine chloride Cardiac rate Sympathetic Parasympathetic

WHILE many experiments have shown the differential behavioral effects elicited by carbachol and norepinephrine injected in the lateral hypothalamus (LHA) of the rat, the differential physiological effects of these drugs has not been thoroughly described. A series of studies were undertaken to investigate the differential effects of both drugs upon several physiological variables and recently Carmona and Slangen [3] have shown that, when injected in the LHA, norepinephrine increased and carbachol decreased the acidity of gastric secretion. The present paper deals now with the effects of those drugs upon frequency and amplitude of the EEG cortical activity and upon cardiac frequency.

It has been shown that substances acting in the transmission of impulses in the peripheral nervous system, are also active and regarded as putative transmitters in the central nervous system. Thirst related behavior in the rat has been found to be controlled by CNS structures transmitting impulses by means of cholinergic substances [4, 6, 7, 8, 16, 17, 20, 21]. Adrenergic substances seem to act as neurotransmitters in neural structures of the rat related to feeding behavior [1, 6, 7, 8, 19]. The injection in the lateral hypothalamus (LHA) of small amounts of carbachol, a drug that is not destroyed by cholinesterase as rapidly as acetylcholine, elicits in water satiated rats drinking behavior. Small amounts of norepinephrine in that same area elicit eating behavior with properties highly similar to behavior caused by food deprivation [7, 8, 9].

Apart from eating and drinking these drugs seem to have also other differential behavioral effects and it has been reported that carbachol in the LHA makes rats agitated and aroused, while norepinephrine injected in the same area makes rats drowsy [15]. In relation to electrophysiological activity, cortical arousal has been reported after intrahypothalamic cholinergic stimulation in mildly anaesthetized rats, concomitant with the inhibition of heart rate [14]. Recently, Buerger *et al.* reported a relationship between neural activity and water ingestion in the rat [2]. In view of these facts, it is of particular interest for behavioral and neurophysiological theories of control of food and water intake, to have quantitative information about the differential effects elicited by adrenergic and cholinergic substances upon EEG cortical activity and upon cardiac frequency in fully conscious animals. The experiments to be described next show that norepinephrine in the LHA elicits a reliable increase in the voltage and a reliable decrease in the frequency of the cortical EEG activity, and that carbachol

[1] This research was supported by grant MH 13189 from the National Institute of Health to Prof. Neal Miller.

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injected in the same area and in the same rats, elicits an opposite effect. While both drugs elicited a drop in the heart rate, carbachol effects were significantly larger.

METHOD

Animals

The animals were 16 male albino rats (Sprague Dawley strain) weighing 300 g.

Implantations and Pretesting

Following the technique developed by Grossman [6], the rats under pentobarbital anaesthesia (40 mg/kg) were stereotaxically implanted with double-walled cannula aimed at the perifornical region in the hypothalamus. De Groot coordinates were: anterior-posterior, 0.0 in relation to bregma, 1.5 lateral and 8.2 mm in depth from the top of the skull [1]. Two jeweler's screws (stainless steel, 8 AL, #46 case screws) implanted in the skull in the parieto-occipital region, 4 mm anterior to lambda and 4 mm lateral to the midline, were used as recording electrodes. A stainless steel braided wire 1 mm in dia. was looped around the skull and used as indifferent electrode. Cannula and electrodes were kept fixed to the skull by acrilic cement. Attached to each screw and to the indifferent electrode were small connectors that protruded from the cemented surface of the skull. After surgery, animals were returned to their home cages where they had access to water and food ad lib.

Five days after surgery, tests for the effects of the chemical stimulation were given, following the technique of Miller, Gottesman and Emery [15]. The animal was placed in a round hardware cloth test cage, 9 in dia. and 7 in high, in which food was placed on the floor and water was available in inverted graduates with standard drinking nozzles placed 1.5 in. from the floor of the cage. After a 15 min habituation period, the animal was taken out of the cage, the food was weighed to the nearest 0.1 g and water was read to the nearest 0.1 ml in the graduated tubes. The rat was held by hand, the inner protective cannula was unscrewed, and the needle of a 10 μl Hamilton microinjection syringe was inserted into the cannula, and 1 μl of the drug was injected. Hereafter the inner cannula was replaced and the rat was returned to the test cage. A cuff made of a little polyethylene tube slipped over the needle of the microsyringe, so that when inserted as far as the cuff would allow, the point of the needle would be flush with the end of the inner cannula. This prevented lesioning the brain with each injection. Sixty min after the injection, the food and water consumptions were measured. After each test, animals were returned to their home cages where they had access to water and food ad lib.

Half of the animals received 1 μl of 40 mM norepinephrine (40×10^{-9} moles) and the next day received 1 μl of 2.4×10^{-9} molar carbachol using NaCl (0.9%) as vehicle. The other half received first carbachol and then norepinephrine. In order to establish a baseline the injections of each drug were repeated 3 times at 48 hr intervals. Rats eating less than 3.5 g of food after the injection of norepinephrine and drinking less than 5 ml of water after carbachol, in the 60 min after the injection, were discarded. Fresh solutions were prepared before each test which was always conducted in the morning. Twenty-four rats were tested to obtain the 16 animals which were to be used in this experiment.

Apparatus

The animal, paralyzed and artificially respirated following the technique developed by Trowill [22], was placed lying on its stomach on a rubber mat in a Grason-Stadler lunch box which had the floor and walls covered with copper screen. The lunch box was placed inside a $3 \times 3 \times 6$ ft long Faraday cage. Electroencephalographic (EEG) and electrocardiographic (EKG) recordings were made on a Grass, Model 5D polygraph. The EEG recording was taken by attaching small connectors from the pins protruding from the cemented skull of the rat, to the input of an a.c. preamplifier (Grass, 5P5). The recording, was bipolar, between both cortical electrodes with the indifferent electrode grounded. The output of the amplifier to the polygraph was used to activate a level detector whenever an EEG wave was above 125 μV. This device and the frequency detector was designed by Mr. Gordon Silverman, E.E., and built in the Electronics Department of The Rockefeller University.

To count the EEG waves the frequency detector used a differentiating circuit to eliminate base line shifts and enhance high frequency spikes from the recorder. The differenciator was connected to a Schmitt Trigger with an adjustable threshold which allowed more suppression of low level signals. This signal triggered a monostable device which produced pulses of constant amplitude and constant duration for each reference level crossing of the input signal. These standard signals were counted using a constant current staircase generator, the magnitude of each step being determined by the frequency trip level. A 1 Hz clock would reset the staircase generator if it did not produce the required number of steps in 1 sec. If the frequency was higher than the reference frequency, the staircase generator triggered a uni-junction transistor which produced a detection signal and reset the 1 Hz clock. The detection signal was fed to a monostable device which energized a relay and this in turn operated a counter.

For recording heart rate (EKG), two subdermal electrodes were inserted under the skin of the left armpit and under the skin of the left hind leg and connected to the a.c. preamplifier of the polygraph. The output of the amplifier to the polygraph pen was used to activate a Schmitt Trigger unit (BRS). The output of the level detector, activated by the EEG waves about 125 μV, and the output of the Schmitt Trigger were fed into a printout counter which counted and printed out in separate channels the number of events per 5 sec every thirty seconds. A BRS programming apparatus controlled the time period and reset the counter.

Procedure

Animals were injected subcutaneously with 3 mg/kg of d-tubocurarine chloride (Squibb) and as soon as they showed signs of difficulty in breathing they were fitted with a face mask and respirated at 1:1 inspiration-expiration ratio 70 times/min with a peak reading of 20 cm of water pressure. The dose injected had been previously found to produce muscle paralysis for 3 hr. In order to rule out the possibility that the effects of the drug might wear off and in turn produce artifacts, a hypodermic needle (24 ga) was inserted under the skin of the right side of the abdominal region after prior injection of Xylocaine (2%) and a solution of 3 mg/ml of d-tubocurarine was constantly infused by means of an electric infusion pump at a rate of

0.15 ml/hr for the duration of the experiment. EEG and EKG electrodes were attached to the animal.

After a period of 30 min during which the experimental room was kept free of noise, animals were randomly assigned to groups N-1 or C-1 with 5 rats in each. The procedure followed was the same for both groups. With a calibration of 75 mV/cm the EEG recording was taken during 15 min in which the counters and programming equipment were turned on and also a baseline was established for EKG. During the next 15 min, the number of EKGs above 125 μV, the number of times the EEG was above 20 cps, and the number of heart beats were automatically counted and printed out during 5 sec periods at 30 sec intervals, with a marker on one of the channels showing the duration of the 5 sec period. Next, programming equipment and polygraph were turned off and the drug was injected. Animals in group N-1 received 1 μl of norepinephrine (40×10^{-9} moles), and those in group C-1 received 1 μl of a carbamylcholine chloride solution (2.4×10^{-9} moles). For the next 45 min (called post-injectional period) EEGs and heart rate (HR) were counted, after which period came another 30 min control period. Animals previously injected with norepinephrine were then injected a second time with carbachol (C-2) and those injected before with carbachol received now norepinephrine (N-2). EEGs and EKGs were computed during the next 45 min. Six rats received sodium chloride injections as first treatment and then half of them received norepinephrine and the other half received carbachol.

At the end of that period animals were injected with a 80 mg/kg dose of Nembutal (pentobarbital sodium) and perfused with physiological saline followed by 10% Formalin. Brains were embedded in paraffin and sectioned serially 50 μ thick and stained according to the technique by Wolf and Yen [23].

RESULTS

Cardiac Changes

The measurements taken 15 min before and 45 min following the intracerebral injections are plotted in Fig. 1. The statistical analysis of the results was made by comparing the scores of the analysis of the results was made by comparing the scores of the 15 min before the injection with the scores of the 15 min afterwards. It was found (Fig. 1-A) that rats injected with carbachol (C-1) showed a significant decrease in HR from a mean control of 420.7 to 350.4 bpm ($t = 67$, $df = 4$, $p<0.001$) with individual p values of 0.001, 0.02 and 0.05. Rats injected with norepinephrine (N-1) also decreased the HR from a mean control of 419.2 to 337 bpm in the 15 min after the injection ($t = 7.05$, $df = 4$, $p<0.001$) with individual p values of

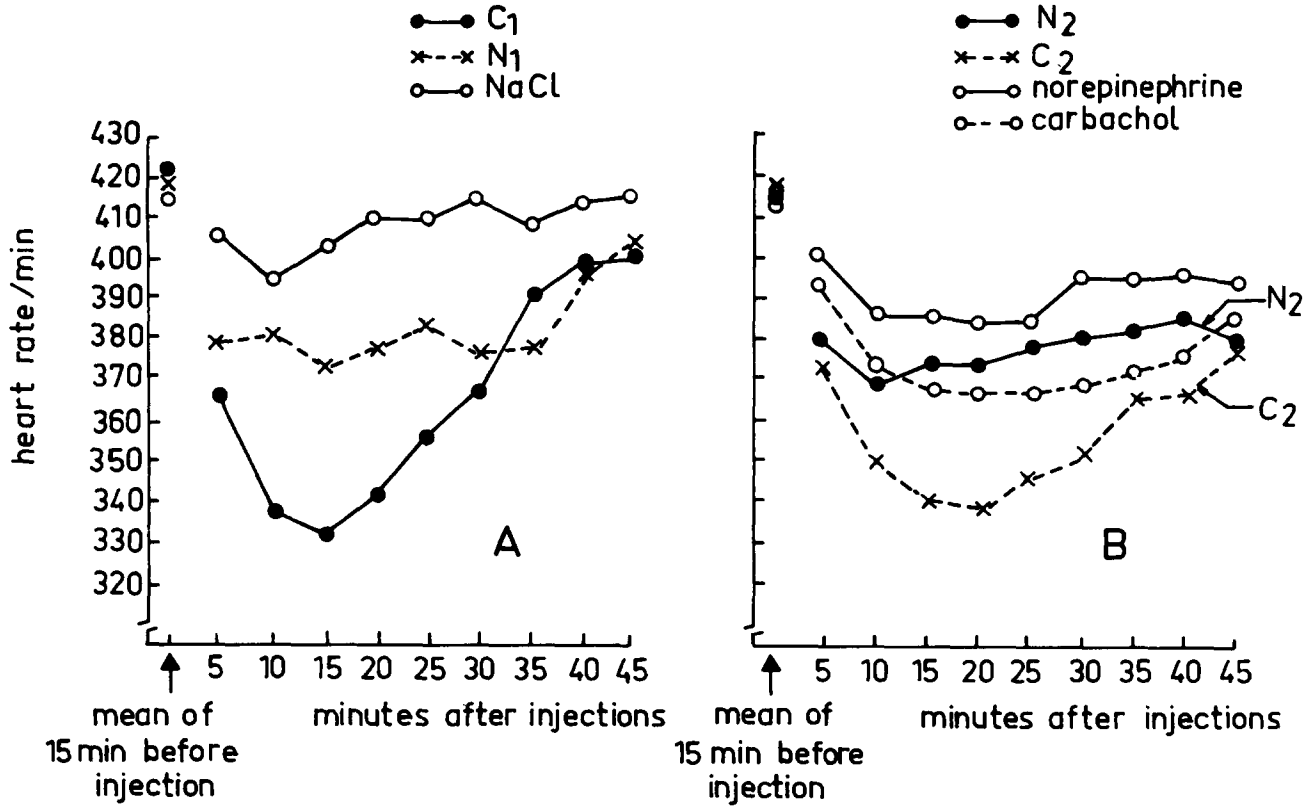

FIG. 1. Effects of LHA injections upon cardiac activity expressed as heart rate/min. (A) While NaCl did not elicit significant changes, carbachol (C-1) elicited a significant decrease in heart rate which reached a maximal effect 15 min after the injection. Norepinephrine (N-1) elicited also a significant decrease in heart rate. (B) The same rats shown in *A* received the other treatment. Rats previously injected with carbachol (C-1) received norepinephrine (N-2) and those injected with norepinephrine (N-1) received an injection of carbachol (C-2). Half of the rats injected in *A* with NaCl received norepinephrine and the other half were injected with carbachol. Notice the magnitude of the effects elicited by the drugs when applied first and as second treatment.

0.001; one rat showed decreases but at the 0.1 level. The HR of both groups was not different before the injections, but 15 min after being injected the group receiving carbachol (C-1) showed a larger decrease in HR than the change elicited in group N-1 by the injection of norepinephrine ($t = 3.84$, $df = 8$, $p<0.001$). In both groups the pre-injection levels were reached about 60 min after the stimulation.

Figure 1-B shows the effects of the reversal of the treatment in the same animals. Seventy-five min after being injected with norepinephrine, rats in group C-2 received carbachol in the LHA; this elicited a change from a mean control of 417.5 to 354.8 bpm ($t = 6.0$, $df = 4$, $p<0.001$). Rats previously injected with carbachol decreased again the HR when receiving norepinephrine (Group N-2) as second treatment ($t = 2.6$, $df = 4$, $p<0.06$) with a mean decrease of 42.12 bpm. Further analysis showed that as a second treatment carbachol elicited a larger decrease in HR than norepinephrine ($t = 2.3$, $df = 8$, $p<0.05$). Comparisons between the effects of carbachol as first treatment with its effects when applied after norepinephrine, showed no differences. The same was found comparing the effects of norepinephrine as first versus second treatment.

In 6 rats injected intrahypothalamically with 1 μl of physiological saline, a non significant decrease in cardiac rate was observed. Seventy-five min after the injection of saline 3 of the rats received carbachol and the other 3 received norepinephrine. A significant decrease in HR occurred after the injection of norepinephrine ($p<0.02$) an effect which was smaller, but not statistically significant, than the effect elicited by norepinephrine in Group N-2. The 3 rats injected with carbachol decreased also the HR ($p<0.02$) and this effect was significantly smaller than the effect elicited by carbachol in Group C-2 ($p<0.05$).

EEG Changes

Amplitude. A clear-cut differential effect upon the amplitude of the EEGs was elicited by the intrahypothalamic injections. When compared with pre-injection levels the increase in the number of EEGs above criterion level was an average 135.3% (52, 269 and 85 percent after 5, 10, and 15 min, respectively) in the 15 min following norepinephrine as first treatment (N-1). In Group N-2, the average increase was 166% (140, 200 and 160 percent at 5, 10 and 15 min respectively) after norepinephrine as second treatment. Carbachol on the contrary, decreased the amplitude of the EEGs and in Group C-1 there was an average decrease of the number of EEGs above criterion level of −23% in the 15 min following the injection (−34, 0 and −34 percent at 5, 10 and 15 minutes). Carbachol as second treatment (C-2) elicited only an average increase of 19.46% (13.20, 13.20 and 32.00 percent) in the 15 min following the injection.

The statistical analysis was performed by comparing for each rat the mean EEG/5 sec above criterion of the measurements taken in the 15 min before the injection with the mean of an equal number of measurements taken in the following 15 min. The 5 rats in Group N-1 increased the number of EEGs above criterion ($p<0.001$ in 3 rats; $p<0.01$ and $p<0.7$) by a mean value of +6.5 compared with the period before the injection ($t = 4.6$, $df = 4$, $p<0.01$). When the same rats (C-2) were injected with carbachol (Fig. 2-B) three rats increased, one rat decreased and the last one showed no change in the amplitude of the EEGs (p values of 0.8, 0.3, 0.9, 0.5 and 0.99, respectively) with a mean value +1.3 ($t = 0.67$, $df = 4$, $p = 0.6$). Comparisons of Group N-1 with C-2 showed a statistically significant larger mean number of EEGs above criterion per 5 sec in Group N-1 ($t = 4.1$, $df = 8$, $p<0.01$).

Rats injected first with carbachol (C-1) showed a non significant decrease in the number of EEGs above criterion in the 15 min period following the injection ($t = 0.75$, $df = 4$, $p<0.5$) (p values of 0.9, 0.4, and 0.001; two rats had increases in the amplitude of the EEGs with $p<0.3$ and 0.05 respectively). The injection of norepinephrine to the same animals (N-2) elicited a mean increase of +3.8 EEGs above the criterion level ($t = 2.5$, $df = 4$, $p = 0.05$). Four rats showed increases ($p<0.001$, $p<0.005$, $p<0.01$ and $p<0.05$) and the other rat showed decreases ($p<0.05$). Comparisons of Group N-2 with Group C-1 showed statistically significant larger amplitudes in N-2 ($t = 2.3$, $df = 8$, $p = 0.05$). It is worthwhile mentioning that Groups C-1 and N-1 did not differ in the period before the drugs were administered as first treatment ($t = 0.8$, $df = 8$, $p = 0.5$) but the difference was quite significant in the 15 min after the injections ($t = 3.27$, $df = 8$, $p<0.01$). Probably due to a carry over effect elicited by the injection of norepinephrine, Group C-2 started with a significantly larger number of EEG above criterion than Group N-2 (Fig. 2-B). This resulted in an overlapping of the curves after the injections and therefore Groups C-2 and N-2 did not differ ($t = 1.6$, $df = 8$, $p = 0.2$), in the 15 min following the injections. Nevertheless, inspection of Fig. 2-B shows that a 420% increase in the number of EEGs above criterion in relation to preinjection levels occurred 25 min after injection. Rats injected with normal saline did not show significant changes in the amplitude of the EEG, but when these same rats were injected with norepinephrine, a significant increase in the amplitude of the EEGs was observed ($p<0.02$). No significant changes occurred after carbachol treatment.

Frequency. The quantitative analysis was performed by comparing 30 measurements of the number of times the EEG frequency was higher than 20 cps in the 15 min before the injections with an equal number of measurements taken in the 15 min afterwards.

All animals in Group C-1 increased the frequency of the cortical EEGs in the 15 min period following the cholinergic stimulation (individual p values of 0.01, 0.02, 0.05, 0.09 and 0.8) and as a group the difference before–after was significant ($t = 3.8$, $df = 4$, $p<0.02$). Animals in Group N-1 on the contrary, had a consistent decrease in the frequency of the EEG after the injection of norepinephrine (p values of 0.01 for 2 rats and 0.05 for the other 3) and as a group the differences between before and after the injection was significant ($t = 5.6$, $df = 4$, $p<0.01$). The differences between Group C-1 and N-1 were statistically significant beyond the 0.01 level ($t = 4.5$, $df = 8$).

The results reversed when the groups received the other treatment and reliable differences appeared between the period before and after the injection. When animals, previously injected with carbachol, received injections of norepinephrine (N-2), a significant decrease in the EEG frequency ($t = 3.6$, $df = 4$, $p = 0.02$) with p values between 0.01 and 0.09 for each rat, was observed. Animals previously injected with norepinephrine showed significant increases ($t = 4.5$, $df = 4$, $p<0.01$) in the cortical EEG frequency after receiving carbachol (C-2) with p values for individual rats at the 0.05 level. The differences between Groups N-2 and C-2 were statistically significant ($t = 3.71$,

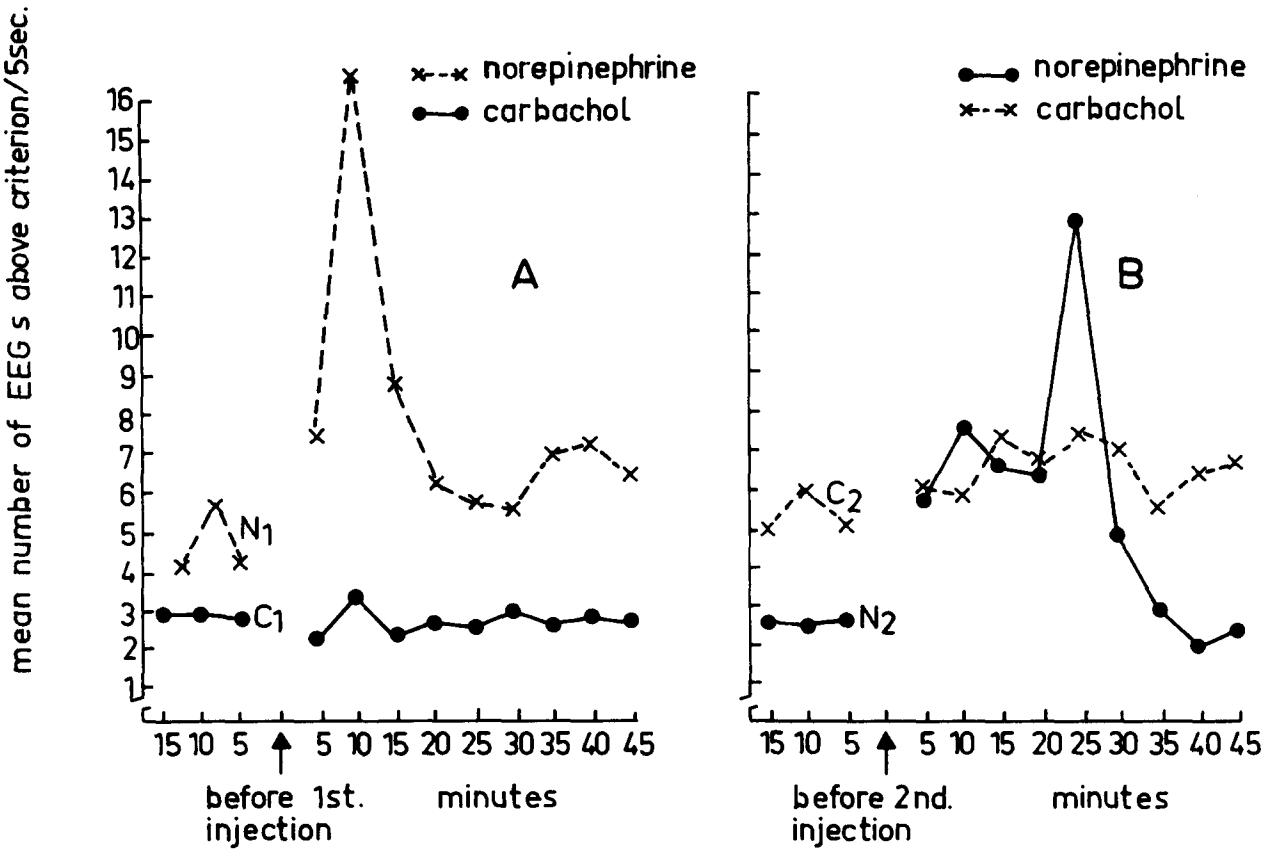

FIG. 2. Shows the effects of LHA injections upon the amplitude of the cortical EEG waves expressed as mean number of EEGs above criterion. (A) Norepinephrine increased the number of EEGs above criterion but carbachol did not. (B) When the same rats previously injected with norepinephrine received carbachol no significant changes occurred. Rats previously injected with carbachol received injections of norepinephrine which elicited a clear increase in the number of EEGs above criterion. Note the peak effects in *A* and *B*.

$df = 8, p<0.01$).

In 6 rats the injection of physiological saline did not elicit EEG changes. In 3 of them non-significant decreases in frequency appeared after injections of norepinephrine ($t = 2.95$, $df = 2$, $p = 0.1$) but in the other 3 rats small but reliable increases in frequency were found after carbachol administration ($t = 4.3$, $df = 2$, $p = 0.05$).

Histological examination showed that the tips of the cannula were in the perifornical region at the level of the medial part of the paraventricular nucleus. There were not enough data to indicate a clear relationship between the results obtained and the location of the injection site.

DISCUSSION

The results obtained in these experiments are clear-cut and indicate that when injected in the LHA both carbachol and norepinephrine elicited bradycardia with larger decreases in heart rate caused by cholinergic stimulation. Also clear differential effects on both amplitude and frequency of EEG cortical activity were observed after intrahypothalamic injection of these drugs.

The statistical analysis performed for the first 15 min after the drugs were injected showed that carbachol elicited a reliable increase in the number of times that the EEG frequency was faster than 20 cps and that norepinephrine elicited the opposite effect, i.e. a reliable decrease in the EEG frequency. These effects on EEG frequency reversed when the rats received the other treatment, indicating that the observed effects were specific for the drugs involved. In Fig. 2 it can be seen that the effects of the drugs upon the amplitude of the EEG were quite the opposite. While carbachol either did not elicit any change at all or elicited decreases in the amplitude of the EEG waves, norepinephrine elicited a large increase in the amplitude of the EEGs, 10 min after the injection. When rats previously injected with carbachol received norepinephrine the peak of the increase in EEG amplitude was observed 25 min after the injection. Again indicating that the observed effects depend on the type of drug administered. Since carbachol caused no change in EEG amplitude in rats previously injected with norepinephrine it can be concluded that the observed effects depend on the type of drug administered.

The difference in latencies of the peak effects seen after the injection of norepinephrine given as first treatment (Fig. 2-A) and when given after carbachol (Fig. 2-B) is difficult to explain. It is not unlikely however that a small amount of a drug remains at the tip of the cannula and is

ejected when a second drug is administered via the same cannula. This can interfere with the main effect of the second drug.

Work by Hernandez-Peon [10] and McPhail and Miller [13] has demonstrated differences between the rat and the cat in response to chemostimulation. Hernandez-Peon *et al.* [11] demonstrated in the cat that injection of either carbachol or acetylcholine along the pathways of a circuit descending from the cortex through the limbic midbrain circuit and ascending from the spinal cord through the medulla and pons to the midbrain, elicited sleep. McPhail however, showed that injecting carbachol in comparable brain structures of the rat elicits EEG arousal. Our results clearly support McPhail's finding and, in addition, they show that norepinephrine elicits the opposite effect namely EEG syncrhonization correlated with an increase in the amplitude of the voltage of the EEGs

Since no information is available on the effects of different drug dosages and also the EEG effects of drug injections at different brain sites are unknown, it is not clear whether the observed effects have anything to do with activation of a neural system specifically at the level of the hypothalamus.

The EEG effects observed after administration of carbachol resemble the EEG reactions induced by external stimuli. This is in agreement with earlier findings in the rabbit [12]. The conspicuous synchronization consistently found after intracerebral administration of norepinephrine is interesting in view of the fact that it is elicited by a drug concentration which also reliably causes eating behavior and an increase in gastric H^+ concentration [3].

Pilot studies performed by the authors have shown that injection of both carbachol and norepinephrine in several other sites of the brain also elicited the same EEG and cardiac changes, e.g. it was found that carbachol and norepinephrine injected into the preoptic area, where norepinephrine does not elicit any eating or drinking but carbachol does elicit drinking, caused EEG arousal and synchronization respectively.

The inhibition of heart rate caused by cholinergic and adrenergic stimulation can be compared with parasympathetic and sympathetic changes caused by electrical stimulation of the hypothalamus. It might be hypothesized that the strong inhibiting effect elicited by carbachol is due to activation of the parasympathetic effects that so easily can be elicited by electrical stimulation of the lateral hypothalamus. The inhibiting effect of norepinephrine we have observed might be caused by activating the hypothalamic sympatico-inhibitory pathway described by Folkow, *et al.* [5]. Although the present as well as our previous study show that parasympathomimetics and sympathomimetics can have differential effects on EEG, heart rate and gastric activity, they do not implicate that the observed effects can be elicited by these drugs only, or by application of these drugs in the lateral hypothalamus only. They do show however, the complexity of effects of chemical stimulation of the brain.

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