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Autonomic Changes Elicited by Chemical Stimulation of Mediodorsal Nucleus of the Thalamus

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POWELL, D. A. AND S. L. BUCHANAN. *Autonomic changes elicited by chemical stimulation of mediodorsal nucleus of the thalamus.* PHARMACOL BIOCHEM BEHAV 25(2) 423–430, 1986.—Chronic indwelling cannulas were implanted in the mediodorsal nucleus (MD) of the thalamus in New Zealand albino rabbits. Heart rate (HR), blood pressure (BP), respiration and electromyographic activity (EMG) were recorded subsequent to the injection of saline, l-glutamate, or carbachol through the previously implanted cannulas. Dose related decreases in HR and increases in BP were obtained after administration of both l-glutamate and carbachol at doses in the micromolar range. Control injections through cannulas chronically implanted in the overlying hippocampus or adjacent ventricles resulted in dramatic HR and BP baseline changes in a parasympathetic direction (viz HR and BP decreases) suggesting that the effects obtained with MD placements were due to chemical stimulation of MD. Responses elicited from MD in the present experiment were similar, but not identical, to those produced by electrical stimulation of MD, suggesting that the latter effects were not due to stimulation of fibers of passage.

Chemical stimulation l-Glutamate Carbachol Mediodorsal N. of the thalamus Blood pressure Heart rate Rabbits

THE mediodorsal (MD) nucleus of the thalamus in the rabbit has been shown to project to a dorsomedial strip of the prefrontal cortex beginning at a mid-callosal level and extending to the frontal pole, wrapping around the frontal convexity, and trailing off along the dorsal border of the rhinal sulcus [1]. Stimulation of this region of prefrontal cortex results in pronounced bradycardia, as well as small depressor responses [4, 7, 18]. These changes mimic the responses elicited by Pavlovian (classical) conditioning contingencies [17]. Stimulation of the more lateral somatomotor and somatosensory prefrontal cortex, which does not receive an MD projection, does not, however, elicit these autonomic changes [7]. Lesions of selected components of this prefrontal system also result in attenuation of classically conditioned bradycardia [4, 5, 18], but lesions of the more lateral isocortex have no effect on classically conditioned bradycardia [5]. However, neither the unconditioned response (UCR) to periorbital electric shock, used as the unconditioned stimulus (UCS), nor the UCR to tones, used as the conditioned stimulus (CS), was attenuated by these lesions [4,18]. These data thus suggest that the MD projection to frontal cortex may be involved in the associative aspects of non-specific autonomic responding associated with classical conditioning contingencies.

A recent series of studies in our laboratory has also been directed at the kinds of autonomic changes elicited by stimulation of the mediodorsal (MD) nucleus proper. West and Benjamin [23] determined that electrical stimulation of MD in the rabbit elicited bradycardia in the anesthetized preparation. Moreover, lesions of the MD prefrontal projection cortex had no effect on this response. We recently determined that stimulation of MD also results in bradycardia accompanied by pressor responses in conscious animals [6]. Thus, although the HR response elicited by MD stimulation is identical to that elicited by stimulation of prefrontal cortex, the BP response is opposite in direction to that elicited by prefrontal stimulation. In this experiment it was also determined that the response elicited from the anteromedial (AM) nucleus resulted in tachycardia and pressor responses [6]. Thus, the response elicited by AM stimulation is different from that elicited by stimulation of MD. However, all thalamic midline nuclei, but not the lateral thalamic group, were cardioactive to electrical stimulation [6].

West and colleagues [22] reported that fibers from the midline nuclei traverse in a lateral direction through MD as fascicles directed toward the more lateral internal capsule. It is thus possible that the results obtained by electrical stimulation of MD are due to stimulation of fibers of passage

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rather than stimulation of the cells of origin of this nucleus. In the present study we attempted to eliminate this possibility by the administration of two drugs known to be putative neurotransmitter agonists through chronically implanted cannulas in MD. Neuronal membrane receptors affected by neurotransmitters are located only on the perikarya, or terminals which affect the perikarya; thus if chemical stimulation has an identical effect to electrical stimulation it may be assumed that electrical stimulation is affecting the cells of origin rather than fibers of passage [9]. In the present experiment, several different doses of l-glutamate, the excitatory amino acid, and the cholinergic agonist carbachol, were administered through chronically implanted cannulas in MD and various evoked atonomic changes measured to assess this possibility.

METHOD

Animals

New Zealand albino rabbits of both sexes were studied. The animals were approximately 150 days old at the time of surgery, and were housed in a climate controlled animal facility with a 07.00–19.00 hr light/dark cycle. All experiments took place during the daylight portion of this cycle. Food and water were available ad lib.

Surgery and Histology

Surgery was performed under ketamine hydrochloride (55 mg/kg IM) and chlorpromazine hydrochloride (10 mg/kg IM) anesthesia using clean surgical techniques. Using a Kopf stereotaxic instrument, a stainless guide cannula (Plastic Products Co., Roanoke, VA) was implanted unilaterally in the mediodorsal nucleus of the thalamus. The stereotaxic coordinates, as determined from the atlas of Sawyer, Everett and Green [19], were P3, L2 and DV 8.0 mm below dura. Prior to implantation a dummy stainless steel stylus was inserted into the guide cannula and its cap screwed to a threaded matching connector on the guide cannula. This stylus was left in place until behavioral testing began. After implantation of the guide cannula containing the stylus, the former was cemented in place with stainless steel screws and dental cement. The animal was then administered 200,000 units of bicillin and allowed to recover for at least one week prior to testing, as described below.

At the completion of testing the animals were sacrificed by a lethal injection of sodium pentobarbital through the ear vein and perfused transcardially with saline followed by 10% formalin solution. The brain was soaked in a 20% glucose/formalin solution for one week. Frozen serial sections of 40 μm through the cannula tracks were taken using an American Optical cryostat. Every fifth section was mounted on slides and stained with thionin. The exact location of the cannula track and tip was then verified by microscopic analysis. Only data from animals in which the cannula tip was verified as being located in the MD were later analyzed.

Apparatus

All animals were restrained in standard rabbit restrainers placed inside a 1×0.5×0.5 m commercial chamber (Industrial Accoustics Co., Bronx, NY) during testing. The experimental contingencies were controlled by a Heath LSI-11 microcomputer supplemented by solid state transistor-transistor logic programming devices. The responses were recorded on a Grass 7D polygraph; these included heart rate (HR), electromyographic activity (EMG), blood pressure (BP) and respiration rate (RR). EMG responses were measured by a Grass model 7P3 preamplifier and integrator, which was set in its integrator mode and calibrated so that a 100 μV change corresponded with a 1 mm deflection of the appropriate oscillograph pen. EMG electrodes consisted of No. 4 insect pins which were acutely inserted into the neck muscle just prior to the experimental session. A previous report [16], which assessed neck EMG, EMG in both the front and hind limbs, and general locomotor activity in restrained rabbits, suggested that the most reliable indicator of movement in this situation is obtained from the neck, since virtually all locomotor changes consist of head movements when rabbits are restrained. The HR was measured by a Grass model 7P4 preamplifier and tachograph set in its tachograph mode. ECG electrodes were stainless steel safety pins inserted underneath the skin on the right front leg and left haunch. BP was measured by a Grass model 7P1 low level DC preamplifier calibrated to translate pen deflections into mm of mercury. Respiration was measured by a Grass model TCT-1R thermocouple inserted into the animal's nostril and connected to a Grass model 7P1 low level DC preamplifier. We have found in previous experiments that the acute and/or chronic insertion of these recording electrodes produces little pain or discomfort in the conscious animal, nor is any infection commonly observed [16,17].

Drug Injections

The drug injections were made through a 28 ga internal cannula which was screwed into the guide cannula at the time of testing. Attached to the internal cannula was a 30 cm length of polyethylene tubing that was connected to a 1.0 μl Hamilton syringe at its distal end. The syringe was attached to a microburet (Micro-metric Instrument Co., Norcross, GA) with a micrometer screw adjustment that permitted delivery of volumes as small as 0.05 μl. Prior to each trial during behavioral testing four different doses of l-glutamate or carbachol were injected in a 0.5 μl volume over a 5.0 sec period. All drugs were prepared fresh in saline on the day of testing. The doses studied were 0, 0.01, 0.10, 1.0, and 10.0 μmol for both drugs. Physiological saline served as the vehicle. In six additional animals 0.5 μl of a solution of 4 μl methylene-blue dissolved in 10 ml of the vehicle solution was injected into MD to assess the degree of diffusion around the cannula tip. Two of these animals were sacrificed immediately after the injection; two were sacrificed 15 sec after the injection and two were sacrificed 30 sec after the injection. The degree of diffusion was determined by gross brain dissection of the thalamus, followed by microscopic examination of the tissue surrounding the cannula and its tip using frozen serial sections through the cannula track and its tip. In four additional animals cannulas were implanted into the hippocampus, and in four animals cannulas were implanted in the lateral ventricles to assess stimulation in these areas to control for possible diffusion of the drug from MD into the surrounding neural structures.

Behavioral Testing

Just prior to testing a 28 ga teflon cannula was inserted into the medial ear artery under local Xylocaine anesthesia for assessment of BP. This catheter was sutured to the surrounding skin and connected to a Statham strain gauge pressure transducer, which was in turn connected to the preamplifier of the polygraph for measurement of BP. Blood

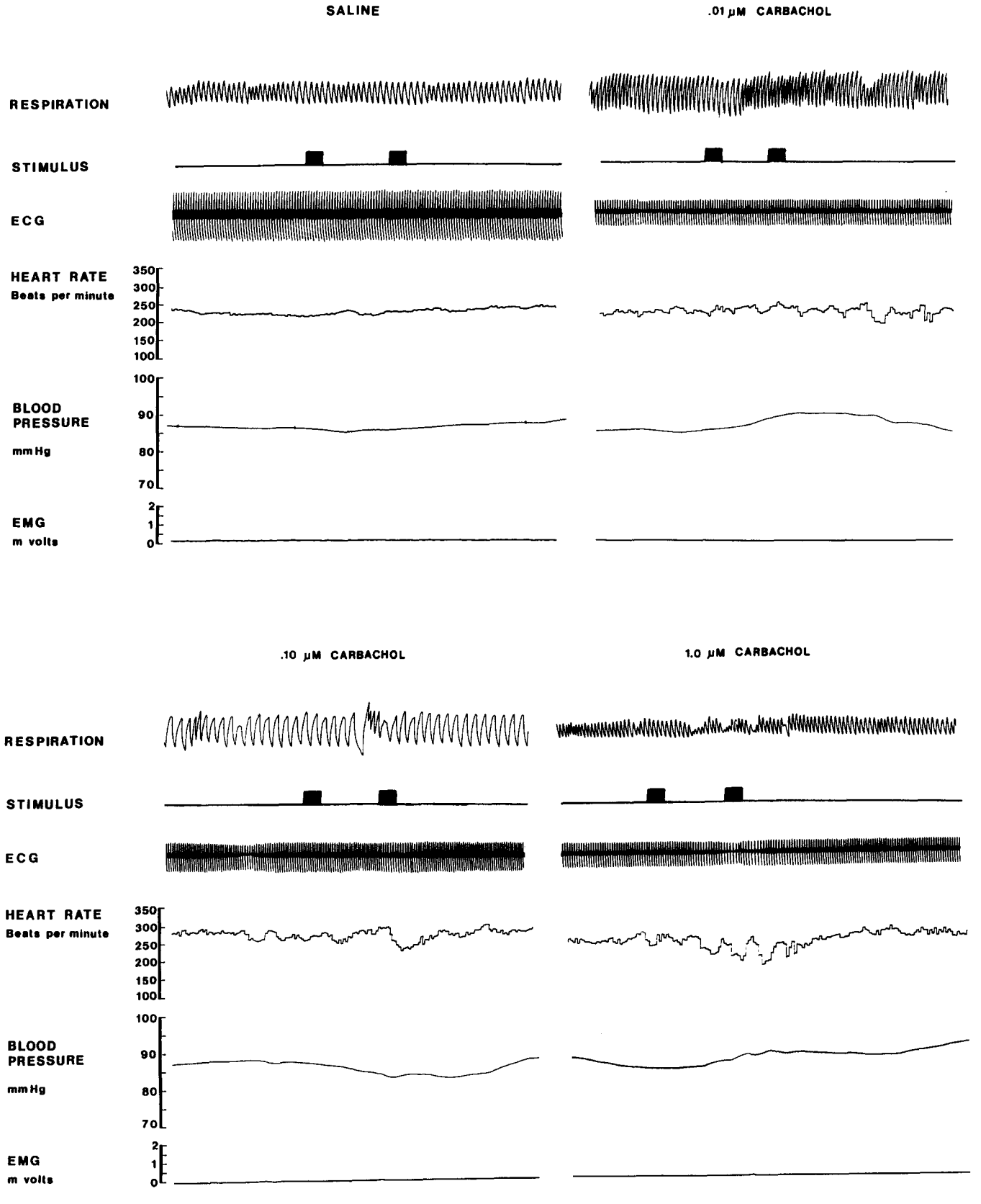

FIG. 1. Cardiovascular changes elicited by different doses of carbachol injected into the mediodorsal N. of the thalamus in a representative animal. The event marks on channel 2 (stimulus) indicate a 5.0 sec period during which the injection was made.

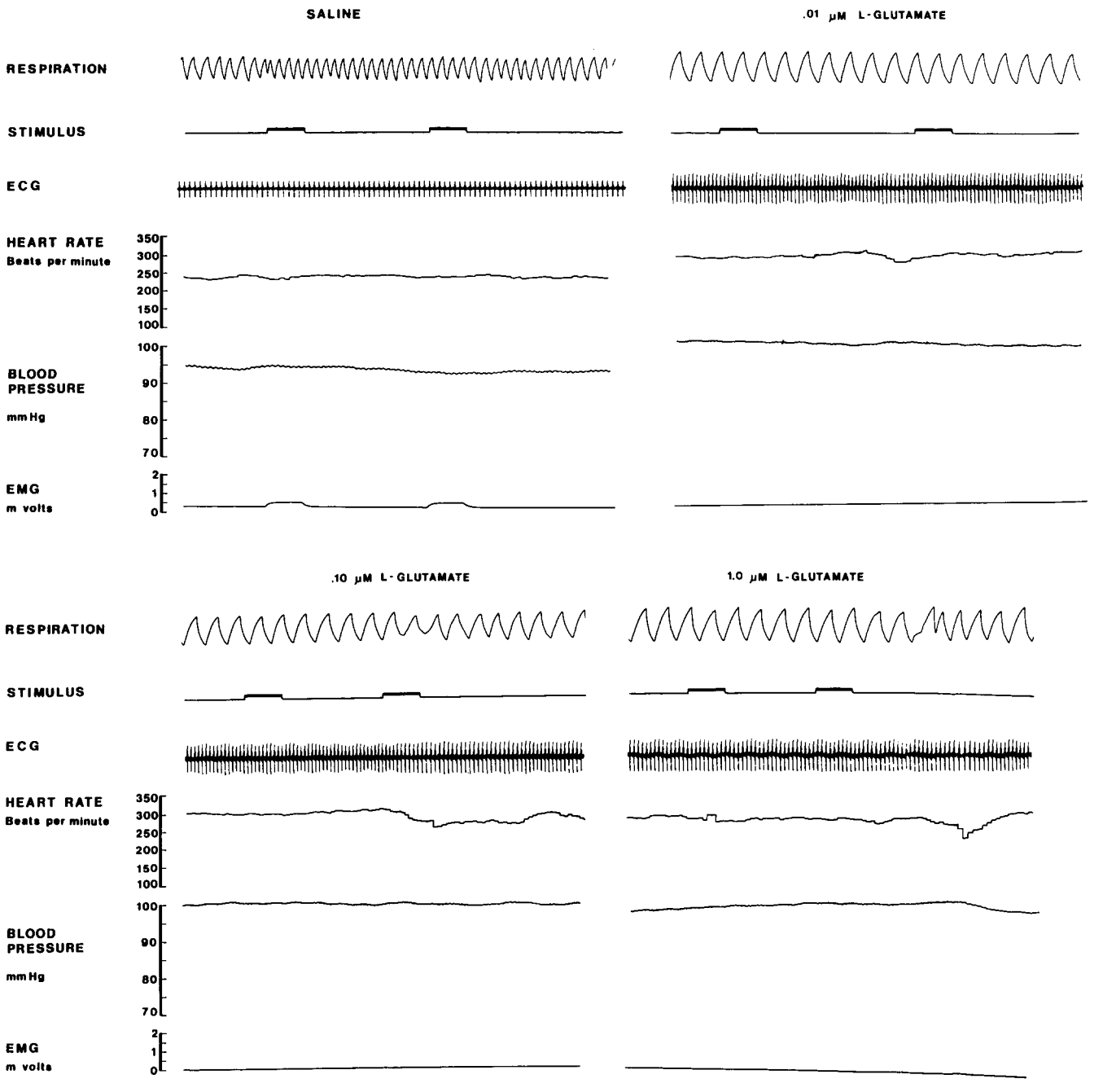

FIG. 2. Cardiovascular changes produced by different doses of l-glutamate injected into the mediodorsal N. of the thalamus in a representative animal. The event marks on channel 2 (stimulus) indicate a 5.0 sec period during which the injection was made.

pressure measurements were recorded in mm of mercury by calibrating the pressure transducer and preamplfier with a mercury manometer. The small lumen and distensibility of the BP cannula precluded measurements of the "true" systolic and diastolic components of the BP wave. Thus the BP changes were an average of these two components (viz mean BP). Although this procedure obviously has the disadvantage that separate measures of diastolic and systolic BP cannot be obtained, it is relatively painless and thus nontraumatizing to the conscious subject. For more details of this procedure see a previous report [17]. During testing only two doses of a given drug, in addition to saline, were administered during a single session in some animals, although in most animals all four doses were examined in a single session. The doses were administered in a predetermined random order in different animals. However, it was found that the administration of higher doses sometimes resulted in a chronic bias of the cardiovascular system in a parasympathetic direction due, no doubt, to diffusion of the drug out of MD and into the cerebrospinal fluid (see below). This observation was unambiguous, however, since baseline heart rate dropped dramatically and BP declined rather than

increased as was observed with MD stimulation (see below). When these changes in HR and BP occurred all further manipulations were delayed until the following day. Thus on the following day the medial ear artery of the remaining ear was cannulated and the remaining doses of the drug were tested. Each dose was replicated two to three times after the first administration. However, multiple presentations of the drug during a single testing period, in some cases, also produced a chronic bias of the cardiovascular system in a parasympathetic direction, thus preventing more than a single replication in some instances. The intertrial interval (ITI) was approximately 5 to 10 min. Mean heart rate was measured for 5 sec prior to stimulation onset; the largest change from this baseline was then recorded for the 5 sec period during injection, and for 10 sec after the injection was completed. These measurements were based on tachograph readings measured to the nearest 0.5 bpm. Mean blood pressure measurements were recorded in mm of mercury at each of the periods referred to above. Respiration frequency was also determined during the pre-, during, and post-injection periods. EMG amplitude on each trial was the maximum voltage recorded during the injection period. All data were analyzed by analysis of variance (ANOVA) with a mixed effects design in which drug served as a non-repeated variable with two levels (viz carbachol vs. l-glutamate); repeated measures variables included dose (5 levels, i.e., saline and four drug dosages) and testing period, (3 levels, i.e., pre-, during, and post-injection periods).

RESULTS

Injection of both l-glutamate and carbachol at μmol doses resulted in decreases in HR and, in many instances, increases in BP, especially at higher doses. These findings are identical to those elicited by electrical stimulation. However, unlike the results of the previous electrical stimulation experiments there were few changes noted in respiration or EMG as a result of chemical stimulation. Data obtained from a representative animal which received carbachol is illustrated in Fig. 1. As can be seen in this figure, a dose-related decrease in HR and a slight increase in BP was obtained as a result of carbachol injections ranging from 0.01 μmol to 1 μmol, although a slight depressor response occurred prior to the pressor response at 0.10 μm. Little change can be seen as a result of saline injections, however. In this instance there were also some indications of RR increases, but these RR changes were not typically obtained. No EMG changes were observed. Similar changes elicited by μmol doses of l-glutamate are shown in Fig. 2 from a representative animal. Again, dose-related decreases in HR and small increases in BP can be observed. As was obtained in most animals the HR change was clearly somewhat larger than the BP change. Indeed in some cases pressor responses failed to occur, although the trend in most animals was for the bradycardiac response to be accompanied by a pressor response. In this case it is also noticable that no RR changes were elicited, especially at lower doses, and no EMG changes occurred.

The mean changes in HR and BP elicited by different doses of carbachol and l-glutamate are shown in Figs. 3 and 4. These represent average HR and BP recorded during the 5 sec pre-injection period, the average greatest change from mean pre-injection baseline occurring during the injection period proper, and the average greatest change from mean pre-injection baseline occurring during the 10 sec post-injection period. These data suggest that l-glutamate and

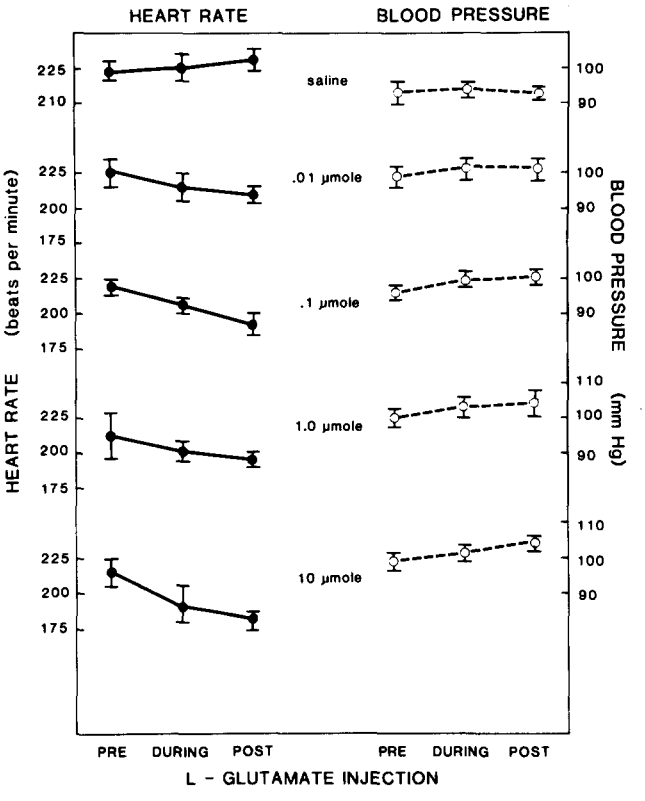

FIG. 3. Mean changes (±1 SEM) in HR and BP elicited by different doses of carbachol injected into the mediodorsal nucleus of the thalamus (n=15). "Pre" represents a mean 5 sec pre-injection baseline period. "During" is the average greatest change from baseline obtained during the 5 sec injection period and "post" is the average greatest change occurring during a 10 sec post-injection period.

carbachol resulted in similar changes in HR and BP. Although little change was elicited by injection of saline in either group, injection of a 0.01 μmol dose of both drugs resulted in a decrease in HR and an increase in BP. As suspected, the HR changes were somewhat larger than the BP changes, although both appeared to be dose-related for both l-glutamate and carbachol.

ANOVA revealed a significant block effect for both the HR and BP changes [$F(2,50)=84.6$, $p<0.001$ and $F(2,46)=53.61$, $p<0.001$, HR and BP, respectively]. Further, there were significant dose × block interactions for both HR and BP [$F(8,118)=15.48$, $p<0.0001$ and $F(8,108)=6.14$, $p<0.0001$, for HR and BP, respectively]. Respiration and EMG changes occurred too infrequently to analyze statistically.

All cannula tips of animals included in the present study were verified by microscopic examination to be located in MD. Moreover, none was closer than 1 mm to the edge of the nucleus in any dimension. Data were discarded from 24 animals which did not meet this criterion. Histological examination of the brains of animals in which methylene-blue was injected revealed that at time periods greater than 15 sec after injection of the drug there was some diffusion of the methylene-blue out of MD and up the cannula track, around the barrel of the guide cannula into the hippocampus. Thus

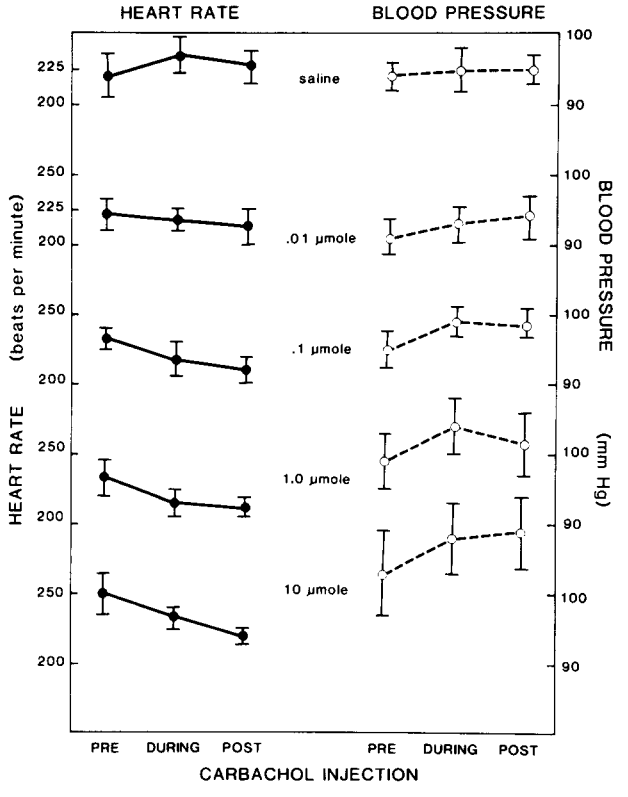

FIG. 4. Mean changes (±1 SEM) in HR and BP elicited by different doses of l-glutamate injected into the mediodorsal nucleus of the thalamus (N=17). "Pre" represents a mean 5 sec pre-injection baseline period. "During" is the average greatest change from baseline obtained during the 5 sec injection period and "post" is the average greatest change occurring during a 10 sec post-injection period.

only changes that occurred within a 10 sec period after injection were included in the present study.

Injection of l-glutamate into the hippocampus at comparable doses to those injected in MD produced a much greater effect than that obtained by administration of this drug into MD. In addition, pronounced baseline changes in a parasympathetic direction occurred in both BP and HR. Administration of l-glutamate into the lateral ventricles at dosages and volumes identical to those administered to MD also produced a very large parasympathetic response in both HR and BP. However, these changes were very unlike the extremely small and discrete changes in HR obtained by administration of these drugs to MD. Moreover the BP change was a substantial depressor response that was quite sustained whereas the BP response elicited by MD stimulation was a pressor response that was much more phasic in nature. An example of l-glutamate injections into (A) left dorsal hippocampus and (B) the sustained parasympathic changes produced by a single 0.50 μl 10.0 μm injection of l-glutamate into the hippocampus in a representative animal is shown in Fig. 5. These autonomic changes are obviously quite different from those observed after MD injections as shown in Figs. 1 and 2. We thus conclude that at the dosage and time parameters utilized in the present experiment, the drugs affected only those cells involved in MD function.

DISCUSSION

It may be concluded from the present experiment that the administration of the putative neurotransmitter agonists, l-glutamate and carbachol, into the mediodorsal nucleus of the thalamus produces autonomic changes that are similar, but not identical, to those obtained by electrical stimulation of MD. These responses consisted of cardiac decelerations accompanied by pressor responses. Moreover, they were dose related and injection of these drugs into closely related structures, i.e., the hippocampus, and lateral ventricles, elicited changes that were quite different from those elicited by MD stimulation. Results of the diffusion studies with methylene-blue also suggest that at the time parameters involved, the cardiovascular responses observed were due to

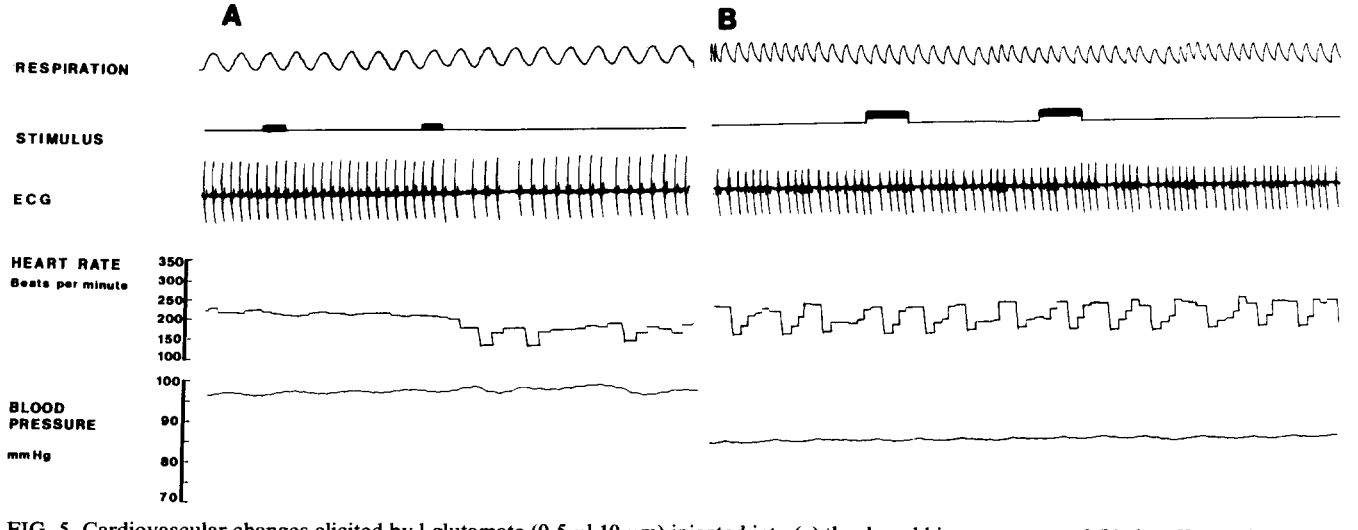

FIG. 5. Cardiovascular changes elicited by l-glutamate (0.5 μl 10 μm) injected into (a) the dorsal hippocampus and (b) the effects of a second similar injection after baseline changes in heart rate and blood pressure had occurred.

chemical stimulation of MD, and not the overlying hippocampus.

It should be noted that the changes obtained by administration of these chemicals into MD were much smaller than those that we have previously obtained by electrical stimulation of this thalamic nucleus [6]. It may also be important that seldom did somatomotor changes occur during the present experiment and respiratory changes were transient at best. Respiratory changes did not occur at the smaller doses at which cardiovascular changes were invariably elicited, and indeed, were never observed in most animals. Both increases in respiration rate and decreases in depth invariably accompanied previous electrical stimulation of MD, although changes in movement (EMG) were not always elicited [6]. Although these differences in electrical and chemical stimulation may be due to stimulation of a different subpopulation of MD cells in the present experiment, compared to those stimulated by electrical stimulation, it is also possible, and indeed highly probable, that electrical stimulation of MD results in stimulation of a considerably larger population of cells than does chemical stimulation.

Although these results were not identical to those produced by electrical stimulation of MD, the fact that autonomic changes are elicited by chemical stimulation of this nucleus suggests that electrically elicited autonomic changes are not solely the result of stimulation of fibers of passage from midline nuclei which pass through MD [22]. It is thus likely that the previously obtained cardiovascular changes elicited by electrical stimulation of MD were the result, at least partially, of stimulation of the axons of cells of origin of this nucleus [6,23]. As noted above, several recent studies have shown that the agranular prefrontal cortex is reciprocally connected to MD [1,13]. Moreover, portions of the agranular prefrontal cortex, as well as the central nucleus of the amygdala, have direct connections to the medullary nucleus tractus solitarious and dorsal motor nucleus of the vagus which control the final common path for cardiovascular and other visceral and autonomic activities [20,21]. Stimulation of each of these areas, as well as other subcortical projection sites of the agranular frontal cortex, produces autonomic activity of a variety of kinds [4,15]. It is thus significant that stimulation of MD also produces bradycardia in the rabbit. However, MD-produced bradycardia is accompanied by pressor rather than depressor responses. Thus the characteristics of MD-elicited autonomic changes, whether produced by electrical or chemical stimulation, differ from those elicited by stimulation of the frontal projection field of MD suggesting that these MD elicited effects may be mediated by other subcortical (perhaps hypothalamic or amygdala) connections. West and colleagues [22] however, report data suggesting that the only subcortical efferent projections of MD in the rabbit are to the caudate nucleus. Connections between MD and thalamic midline nuclei, which are also cardioactive to electrical stimulation [6], thus might mediate a diencephalic autonomic response of the type evoked by MD stimulation [3].

An important question which has also concerned us is whether the EMG and respiratory changes concomitantly elicited from MD might mediate the bradycardia and depressor responses elicited by electrical stimulation of MD as well as MD projection cortex. The present data suggest that this is unlikely to be the case, since, in almost all instances, and especially at lower dosages, bradycardia and pressor responses were obtained without accompanying changes in movement or respiration.

It has been repeatedly shown that the MD nucleus of the thalamus is intimately involved in learning and memory in species ranging from man to rats; see [14] for a recent review. Prefrontal cortex has also been linked to learning and memory [2, 4, 8, 10, 18]. The findings of the present study, combined with these neuroanatomical and electrophysiological studies, suggest that the mediodorsal nucleus of the thalamus and its projection cortex may provide a substrate for the autonomic accompaniments of associative learning.

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