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$GABA_A$ and excitatory amino acid receptors in dorsomedial hypothalamus and heart rate in rats

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We have previously shown that microinjection of drugs that interefere with the function of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) into the hypothalamus produces cardiorespiratory and behavioral changes resembling those seen in emotional stress. The purpose of this study was to determine whether excitatory amino acids (EAAs) can produce a cardiovascular response similar to that caused by the $GABA_A$ receptor antagonist bicuculline methiodide (BMI) when microinjected at the same hypothalamic site in urethan-anesthetized rats and to clarify the precise locus of action of these agents. *N*-methyl-D-aspartic acid (NMDA, 0.68-6.8 pmol/50 nl) and kainic acid (KA, 0.47-4.7 pmol/50 nl) produced dose-related increases in heart rate and blood pressure when injected at sites in the dorsomedial hypothalamus reactive to BMI (20 pmol/50 nl). Higher doses of NMDA (68 pmol), however, failed to elicit consistent increases in heart rate and blood pressure when injected at these same sites. The effects of NMDA were selectively blocked by the NMDA receptor antagonist 2-amino-5 phosphonopentanoic acid, whereas the effects of KA were selectively blocked by the non NMDA EAA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3dione. These results demonstrate that *1*) blockade of inhibitory amino acid receptors or stimulation of EAA receptors in the dorsomedial nucleus of the hypothalamus produces tachycardic and pressor responsesin urethan-anesthetized rats and 2) use of high doses of EAAs may be an unreliable method of evoking local neuronal excitation in certain regions of the central nervous system.

Historically, the hypothalamus has been implicated in playing an important role in the autonomic and behavioral responses to emotional stress. Electrical stimulation of the hypothalamus evokes a characteristic pattern of changes that includes increases in blood pressure and heart rate as well as behavioral effects (17,34). However, the utility of electrical stimulation studies in mapping the central nervous system is questionable because electrical stimulation excites all local neural elements including fibers of passage. Thus interpretation of these studies is uncertain because the observed responses may result from activation of local neurons or axons projecting through the area of stimulation (21). A more recently developed technique to evoke local excitation of neurons involves the use of excitatory amino acids (EAAs). This approach, now widely employed, is thought to be advantageous over electrical stimulation

because EAAs are capable of exciting most if not all neurons in the mammalian central nervous system without affecting fibers of passage (11).

Recent studies have tested the hypothesis that, at hypothalamic sites at which electrical stimulation elicits increases in blood pressure and heart rate, chemical stimulation will produce similar responses. Microinjection of molar concentrations of either glutamate or *dl*-homocysteic acid (DLH) at various hypothalamic sites failed to produce consistent increases in both heart rate and blood pressure (4, 10, 12, 27). Therefore, these investigators concluded that excitation of hypothalamic neurons does not result in tachycardia and hypertension and that some of the changes seen with electrical stimulation were the result of activating fibers of passage. Yet, other microinjection studies have demonstrated that prostaglandin E_2 (9), α-melanocyte-stimulating hormone (8), or drugs that interfere with the function of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (6, 7, 32, 33) can act within the hypothalamus to produce changes in cardiovascular function similar to those seen with electrical stimulation, i.e., tachycardia and hypertension. Furthermore, removal of the functional activity of neurons at these same sites by either microinjecting the $GABA_A$ receptor agonist muscimol (16) or electrolytic lesioning (26) prevents the tachycardia and hypertension associated with stress. These latter studies therefore suggest that activation of neurons in the hypothalamus does, indeed, result in increases in heart rate and blood pressure and that these same neurons may play an important role in mediating the cardiovascular responses to experimental stress.

The conflicting results of studies involving intrahypothalamic microinjection of EAAs and other drugs such as the GABA antagonists might be explained in at least two ways. First, glutamate and DLH may have been injected at hypothalamic sites other than those at which GABAergic drugs act to produce cardiovascular effects. Because previous studies involving intrahypothalamic injection of GABA antagonists usually employed relatively large volumes (0.25-l μl), the exact site of action of these agents may be in question. This uncertainty is further compounded by an apparent error, recently highlighted by Spencer and colleagues (27), regarding the delineation of the borders of the posterior hypothalamic nucleus and the dorsomedial nucleus of the hypothalamus in the atlas of Pellegrino et al. (20). Second, the injected doses of these EAAs may have been so high as to elicit depression rather than excitation of local neural activity, a phenomenon that has been shown to occur after injection of molar concentrations of EAAs in other areas of the central nervous system (15). To test these possibilities, we microinjected picomolar doses of the relatively selective EAA receptor agonists *N*-methyl-D-aspartate (NMDA) and kainic acid (KA) in volumes of 50 nl (μm concentrations) at hypothalamic sites shown to be reactive to the $GABA_A$ receptor antagonist bicuculline methiodide (BMI) in urethan-anesthetized rats. In addition, the EAA receptor

antagonists 2-amino-5 phosphonopentanoic acid (AP5), thought to be selective for the NMDA subtype of EAA receptor (5), and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), an agent exhibiting dose-related selectivity for non-NMDA EAA receptors (31), were employed to determine the role of specific EAA receptor subtypes in generating the effects of each agonist.

METHODS

Male Sprague-Dawley rats (250-350 g, Taconic Farms) were anesthetized with urethan (1.35 g/kg ip). The left femoral artery and vein were cannulated with PE-50 tubing filled with heparinized saline. The arterial line was connected to a pressure transducer for the direct measurement of arterial pressure with the pulse pressure signal triggering a cardiotachometer for the measurement of heart rate. Arterial pressure and heart rate were continuously recorded on a Beckman 511A strip chart recorder while respiratory rate was visually monitored and recorded at appropriate times. Rectal temperature was monitored and maintained at $37 \pm 0.5^{\circ}C$.

Microinjections were made using glass micropipettes. Glass capillary tubing was pulled, broken back, and then bevelled to a smooth tip with an outer diameter of 50-100 μm. The micropipette was then attached to a length of PE-10 tubing, and the system was filled with mineral oil and water to create an oil-water interface visible with low-power magnification. The tubing was then connected to a water-filled 10-μ1 Hamilton syringe mounted in a manual micrometer drive. The micropipette was loaded by placing the oil-filled tip in a drug solution and then slowly pulling back on the syringe plunger. The drug solution was delivered by slowly advancing the plunger with the manual micrometer drive. The distance the interface traveled, as viewed through a stereoscope with an ocular micrometer scale, was directly proportional to the volume delivered. Calibration studies verified the accuracy and reproducibility of this system for the delivery of our standard microinjection volume of 50 nl. All microinjections were unilateral (right side) and consisted of a total volume of 50 nl infused over 15-20 s. The micropipette remained in place for 1 min after ending the infusion.

Hypothalamic sites where microinjection of the $GABA_A$ receptor antagonist BMI elicited short-latency tachycardia were determined as in our previous studies (6, 7, 23-25, 33). The upper incisor bar was adjusted to 5.0 mm above the interaural plane. With bregma serving as the reference point, the micropipette was lowered to the target coordinates at a 10° angle with respect to the sagittal plane to avoid damaging the midsagittal sinus. The stereotaxic coordinates for the target were posterior 1.2, right 0.5, depth -8.7 corresponding to the posterior hypothalamic nucleus according to the atlas of Pellegrino et al. (20). In early

experiments (dose-response relationship of NMDA and KA), proper placement of the micropipette was verified by injecting 20 pmol BMI, and only those sites at which BMI produced an increase in heart rate of ≥90 beats/min with an onset of ≤30 s from the beginning of the infusion were examined further. In later experiments (using NMDA and non-NMDA EAA receptor antagonists), proper placement of the pipette was verified by injecting either 6.8 pmol NMDA or 2.3 pmol KA; only those sites at which the EAA produced an increase in heart rate of ≥50 beats/min with an onset of ≤30 s from the beginning of the infusion were studied. If the change in heart rate failed to meet these criteria, the HD or RL coordinates were altered by 0.3 mm, and the new placement was tested until an active site was located. Once an active site was located, the experiment proceeded with typically three or four subsequent injections spaced in time such that any effects on heart rate caused by the previous injection had dissipated before the next injection.

In early experiments, the injection site was marked with the dye 2,3,5-triphenyltetrazolium chloride (100 ng/50 nl, Aldrich). The animal was immediately killed, and the brain was removed and frozen at -80°C. Later, coronal sections (50 μm) were cut on a freezing microtome and compared with the atlas of Pellegrino et al. (20) for verification of the injection site. In later experiments, the injection site was marked by microinjecting 50 nl of a solution containing india ink diluted 1:l with saline. The pipette remained in place for 10 min at which time it was slowly removed. The animal was immediately perfused with 50 ml of saline followed by 500 ml of fixative (1.25% glutaraldehyde, 1% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4) over 30-40 min. The brain was removed and stored in the fixative for l-3 days. Coronal sections (40 μm) were cut on a freezing microtome, mounted on gelatin-coated slides, and stained with a 1% neutral red solution. Location of injection sites was then determined according to the atlas of Konig and Klippel (14) and also the atlas of Pellegrino et al. (20).

Drugs used in these experiments included urethane, decamethonium bromide, BMI, NMDA, KA, *dl*-2-amino-5-phosphonopentanoic acid (all purchased from Sigma Chemical, St. Louis, MO) and CNQX (Tocris Neuramin, UK). All drugs were dissolved in 0.9% saline solution with the final pH adjusted to 6.0-7.5.

Results are expressed as means ± SE. The data were analyzed by linear regression, analysis of variance, and paired *t* tests. The 5% limits of probability were accepted as significant.

RESULTS

Microinjection of 2-20 pmol BMI into the dorsomedial hypothalamus caused dose-related ($P < 0.05$, by linear regression) increases in heart rate and blood pressure (Table 1, Figs. 1 and 2). These changes began within 5-30 s from the start of the infusion and reached a peak effect in 3-6 min. Microinjection of NMDA (Fig. 1, *B-E*) or KA (Fig. 2, *B-D*) at hypothalamic sites reactive to BMI produced cardiovascular changes similar to those produced by BMI. Both NMDA (0.68-6.8 pmol) and KA (0.47-4.7 pmol) produced dose-related ($P < 0.05$, by linear regression) increases in heart rate and blood pressure (Table 1, Figs. 1 and 2). However, the cardiovascular response to microinjection of higher doses of NMDA was diminished or occasionally absent (20 and 68 pmol, Fig. 1, *E* and *F*). In a series of five experiments in which injections of 6.8 pmol NMDA produced marked increases in heart rate (+71 ± 9 beats/min) and modest elevations in blood pressure (+9 ± 1 mmHg), injections of 68 pmol NMDA at the same site failed to elevate heart rate (+24 ± 21 beats/min) or blood pressure (+3 ± 3 mmHg) consistently. A similar phenomenon was not apparent with KA. However, because of the longer time for recovery and possible excitotoxicity associated with this agent, higher doses of KA were not systematically tested. The cardiovascular effects produced by microinjection of either 20 pmol BMI, 6.8 pmol NMDA, or 2.3 pmol KA were reproducible over four successive injections. Injection of the saline vehicle at sites reactive to BMI and the EAAs did not produce significant changes from baseline in heart rate or blood pressure (Table 1).

In addition to the cardiovascular effects, 20 pmol BMI, 6.8 pmol NMDA, and 4.7 pmol KA produced marked increases in respiratory rate estimated at 40-90 breaths/min above resting baseline values. To determine whether the changes in heart rate were secondary to the increases in respiratory rate, the tachycardia produced by 6.8 pmol NMDA was quantitated before and after elimination of respiratory changes by neuromuscular paralysis (decamethonium bromide, 2 mg/kg iv) and artificial respiration with room air in three rats. The increase in heart rate before (+65 ± 8 beats/min) and after (+67 ± 15 beats/min) paralysis was not different ($P > 0.05$, paired *t* test). Previous work has demonstrated that the tachytachycardic response to microinjection of BMI at this hypothalamic site is also independent of changes in respiration (6).

To determine the role of different subtypes of EAA receptors in mediating the cardiovascular responses to microinjection of the EAA agonists, a series of experiments were undertaken using the NMDA receptor antagonist AP5 and the purported non-NMDA EAA receptor antagonist CNQX. In these experiments, the response to microinjection of either 6.8 pmol NMDA or 2.3 pmol KA in the presence of AP5 or CNQX was examined once the site was shown to be responsive to the agonist alone. Doses of the antagonists (AP5 or CNQX) that would be both selective and effective for a given EAA receptor subtype were established. Thus AP5 (5-50 pmol) blocked, in a dose-related ($P < 0.05$, by linear regression) and

reversible fashion, the cardiovascular effects of NMDA without significantly affecting the response to microinjection of KA (Fig. 3). In a parallel series of experiments, 25 pmol CNQX markedly and reversibly attenuated the cardiovascular effects of KA without significantly affecting the response to NMDA (Fig. 4). Larger doses of CNQX (50 and 100 pmol) effectively antagonized the response caused by either EAA (data not shown). Microinjection of either 50 pmol AP5 or 25 pmol CNQX alone had no significant effect on basal heart rate (maximal changes from baseline within 10 min after injection; +5 ± 3 and -5 ± 3 beats/min, respectively; $n = 3$) or blood pressure (+l ± 1 and -1 ± 1 mmHg, respectively; $n = 3$; $P > 0.05$).

In the experiments described above, subsequent histology using india ink and fixed brains (see METHODS) indicated that the sites reactive to NMDA were localized to an area bounded laterally and dorsoventrally by the fornix and mammillothalamic tract and medially by the third ventricle (Fig. 5). Injection of 6.8 pmol NMDA 0.5 mm lateral, dorsal, or posterior to a reactive site typically resulted in increases in heart rate of <20 beats/min. In 3 of the 11 experiments summarized in Fig. 5, 20 pmol BMI was microinjected at a site defined as reactive to NMDA and produced increases in heart rate >90 beats/min. According to the atlas of Konig and Klippel (14) or Paxinos and Watson (19), the region of the greatest reactivity to NMDA corresponded to the dorsomedial hypothalamic nucleus (Fig. 6A).

DISCUSSION

The results of this study provide clear evidence that the EAA receptor agonists NMDA and KA produce an increase in heart rate and blood pressure similar to that produced by the $GABA_A$ receptor antagonist BMI when microinjected at the same site in the dorsomedial hypothalamic nucleus of anesthetized rats. BMI, NMDA, and KA produced, in a dose-related manner, marked increases in heart rate with modest elevations in blood pressure. In earlier studies, we demonstrated that the cardiovascular changes caused by intrahypothalamic injection of BMI were associated with an increase in splanchnic sympathetic nerve activity (33) and that the tachycardia could be prevented by the ,β-receptor antagonist propranolol or by ganglionic blockade (6). These studies would, therefore, indicate that the changes in cardiovascular function were mediated by an increase in the activity of the sympathetic nervous system. Consequently, the coincident site of injection and the similar pattern and onset of cardiovascular changes in the present study suggest that the EAA agonists NMDA and KA and the GABA antagonist BMI are activating the same cardiovascular effects.

To determine whether microinjection of NMDA and KA were, indeed, mediated by specific EAA receptor subtypes, the EAA receptor antagonists AP5 and CNQX were used. AP5 has been identified as a

competitive antagonist selective for the NMDA receptor subtype (5,31), whereas, more recently, CNQX has been shown to be a competitive antagonist at non-NMDA EAA receptors (13, 31). However, the selectivity of CNQX for the non-NMDA EAA receptors is uncertain because CNQX has been shown to antagonize NMDA receptor mediated responses by blocking the glycine regulatory site associated with this receptor complex (2, 31). Therefore, because the selectivity of any drug is a function of its dose or concentration, we determined a dose of each antagonist that was effective and selective. In these studies, the antagonist was delivered in the same solution as the agonist. Concomitant administration was thought to be advantageous over an antagonist pretreatment protocol because it would *1*) reduce the number of injections each animal received in the course of an experiment and *2*) possibly allow for the use of lower and, therefore, more selective doses of the antagonists, since larger doses given as a pretreatment might be required to maintain an effective local concentration until injection of the agonist. Coinjection of AP5 eliminated the cardiovascular response to microinjection of NDMA at a dose (50 pmol) that did not significantly decrease the response to KA. In a parallel series of experiments, CNQX markedly attenuated the cardiovascular response to KA at a dose (25 pmol) that did not affect the response to NMDA. Higher doses of CNQX (50 and 100 pmol) eliminated the response to KA but also significantly blunted the response to NMDA. Interestingly, when either AP5 or CNQX was microinjected alone at sites previously shown to be cardioreactive to either NMDA or KA, neither antagonist produced significant changes in heart rate, blood pressure, nor respiratory rate from baseline. This would suggest that in urethan-anesthetized rats no EAA receptor-mediated activity of the neurons sensitive to NMDA or KA is detectable. Conversely, microinjection of the $GABA_A$ receptor agonist muscimol at sites reactive to the antagonist BMI also failed to produce significant reductions in heart rate or blood pressure in urethan-anesthetized rats (32). These findings suggest that hypothalamic neurons capable of generating tachycardia and hypertension when activated by blockade of inhibitory amino acid receptors or stimulation of EAA receptors are quiescent and, therefore, do not contribute to the maintenance of basal heart rate or blood pressure under these experimental conditions.

Histology from this study indicates that the area responsive to BMI and the EAAs NMDA and KA is localized to the dorsomedial nucleus of the hypothalamus. Previously reported work from this lab (6, 7, 16, 23-25, 33) suggested that the area responsive to GABAergic drugs was localized to the posterior hypothalamus. The reason for this apparent inconsistency resides in our reliance before this study exclusively on the atlas of Pellegrino et al. (20) which, as noted recently by others (27), incorrectly extends the anterior border of the posterior hypothalamic nucleus well into the tuberal hypothalamus. Consequently, much of the region labeled as the posterior hypothalamic nucleus in Pellegrino et al. (20) actually represents the dorsomedial nucleus of the hypothalamus according to both Paxinos and Watson (19) and Konig and

Klippel (14) (see Figs. 5 and 6). Comparison of figures from our previous studies with either of these latter references indicates clearly that the dorsomedial nucleus corresponds to the location of virtually all previously localized injection sites (see Fig. 5 in Ref. 6; Fig. 1 in Refs. 23-25). Therefore, the cardiovascular effects caused by intrahypothalamic microinjection of GABAergic drugs as previously reported were evidently mediated by neurons originating in the dorsomedial nucleus of the hypothalamus. This conclusion is significant in light of our previous work because the dorsomedial nucleus projects to a number of central nervous system sites involved in the control of autonomic nervous system activity (28-30) and has been suggested to play an important integrative role in mediating limbic influences on cardiovascular function (1).

Recently, microinjection of KA into the paraventricular nucleus was reported to result in increases in heart rate and blood pressure similar to those seen in the present study (22). However, the high doses of KA (140-4,700 pmol) used in those experiments presents the possibility of the drug diffusing to and acting at other sites in the central nervous system to produce these cardiovascular effects. Although the present study did not directly measure the extent of the diffusion of the drug from its site of injection, the relatively low doses and small injection volumes employed and the distribution of reactive sites suggests that the action of these drugs is likely to be within the dorsomedial nucleus.

The present study demonstrated that intrahypothalamic injection of micromolar concentrations of EAAs can elicit both hypertension and tachycardia. Our results contrast with the work of others in which microinjection of 0.15-1.0 M solutions of the relatively nonselective EAAs glutamate or DLH at sites in the hypothalamus of the rat failed to elicit any significant effects on heart rate and blood pressure (4, 10) or produced minimal increases in heart rate accompanied by a fall in blood pressure (12). The conclusion of these previous studies, that activation of neurons in the dorsomedial hypothalamus does not result in increases in heart rate and blood pressure, was based on the assumption that molar concentrations of EAAs can excite virtually all neurons, an assumption that underlies the utility of EAAs as tools to elicit local neuronal excitation (11). However, Lipski and colleagues (15) demonstrated that although lower concentrations elicited the expected increases in local unit activity, microinjection of 0.5-l M solutions (lo-150 nl) of either glutamate or DLH failed to produce long-lasting activation as would have been predicted. Instead, neurons in the vicinity of the injection site (0-500 μm) showed, after a brief period of excitation, a long-lasting depression of activity. In a parallel fashion, we were able to demonstrate maximal cardiovascular effects with NMDA and KA at concentrations of 100-150 μM [and similar injection volumes (50 vs. 10-150 nl)], whereas microinjection of NMDA at a concentration of 1.5 mM or greater at the same sites produced variable effects on cardiovascular function. It is possible that these higher concentrations of

NMDA were *1*) producing a depolarizing blockade of the target neurons as was suggested by Lipski and colleagues (15) and McAllen and colleagues (18), 2) diffusing to other sites to elicit changes that are opposite or inhibitory to the observed effects, or *3*) activating inhibitory interneurons at the site of injection that are not activated by low concentrations of NMDA. Regardless of the precise mechanism responsible, the failure to demonstrate increases in heart rate and blood pressure on microinjection of EAAs into the dorsomedial hypothalamus in previous studies was probably related to the unreliable patterns of local excitation associated with use of molar concentrations of EAAs.

In summary, the present study presents data that are in disagreement with recent reports suggesting that chemical activation of cell bodies in the hypothalamus cannot generate increases in heart rate and blood pressure (4, 10, 12). The data from our study, however, provide clear evidence that blockade of $GABA_A$ receptors or stimulation of NMDA or non-NMDA EAA receptors in the dorsomedial nucleus of the hypothalamus produces marked tachycardia and significant increases in blood pressure in the urethan-anesthetized rat. Our results also suggest that the technique of evoking local excitation of neurons by the indiscriminate injection of a single high concentration of EAAs may have serious limitations at certain sites in the central nervous system.

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TABLE 1. Cardiovascular effects caused by microinjection of BMI, NMDA, KA, or saline into dorsomedial hypothalamus in anesthetized rats

	Baseline		Maximum Change		Time to	
	HR, beats/min	BP, mmHg	HR, beats/min	BP, mm	Max HR, Recovery, min	50% Recovery, min
Saline	400±20	99±1	+8±3	+2±1		
BMI						
2 pmol	379±10	99±2	+18±2*	+4±2	3.0±0.2	8.1±1.0
10 pmol	364±15	98±5	+69±13*	+6±2*	4.1±0.4	9.5±0.7
20 pmol	363±12	103±3	+105± 7*	+17±3*	4.5±0.6	15±2.0
NMDA						
0.68 pmol	351±19	103±2	+13±1*	-3±1	0.7±0.2	1.7±0.2
2.0 pmol	371±16	104±4	+36±6*	+5±2	0.9±0.1	2.7±0.3
6.8 pmol	375±15	106±3	+65±5*	+7±1*	1.1±0.1	3.4±0.2
KA						
0.47 pmol	380±8	88±3	+25±4*	+4±1*	1.7±0.3	4.5±1.0
1.4pmol	381±12	104±4	+45±5*	+7±1*	2.5±0.5	9.0±0.9
4.7 pmol	384±21	103±4	+73±8*	+13±2*	3.6±0.3	36±4.0

Values are means ± SE ($n = 4$ for BMI, NMDA, KA, and saline). HR, heart rate; BP, blood pressure; see text for additional definitions for abbreviations. BMI (20 pmol) produced increases in HR and BP of 115 ± 9 beats/min and 13 ± 2 mmHg ($n = 8$), respectively, in group of rats injected with either NMDA or KA.* Significant change from baseline by paired t test ($P < 0.05$). See text for abbreviations.

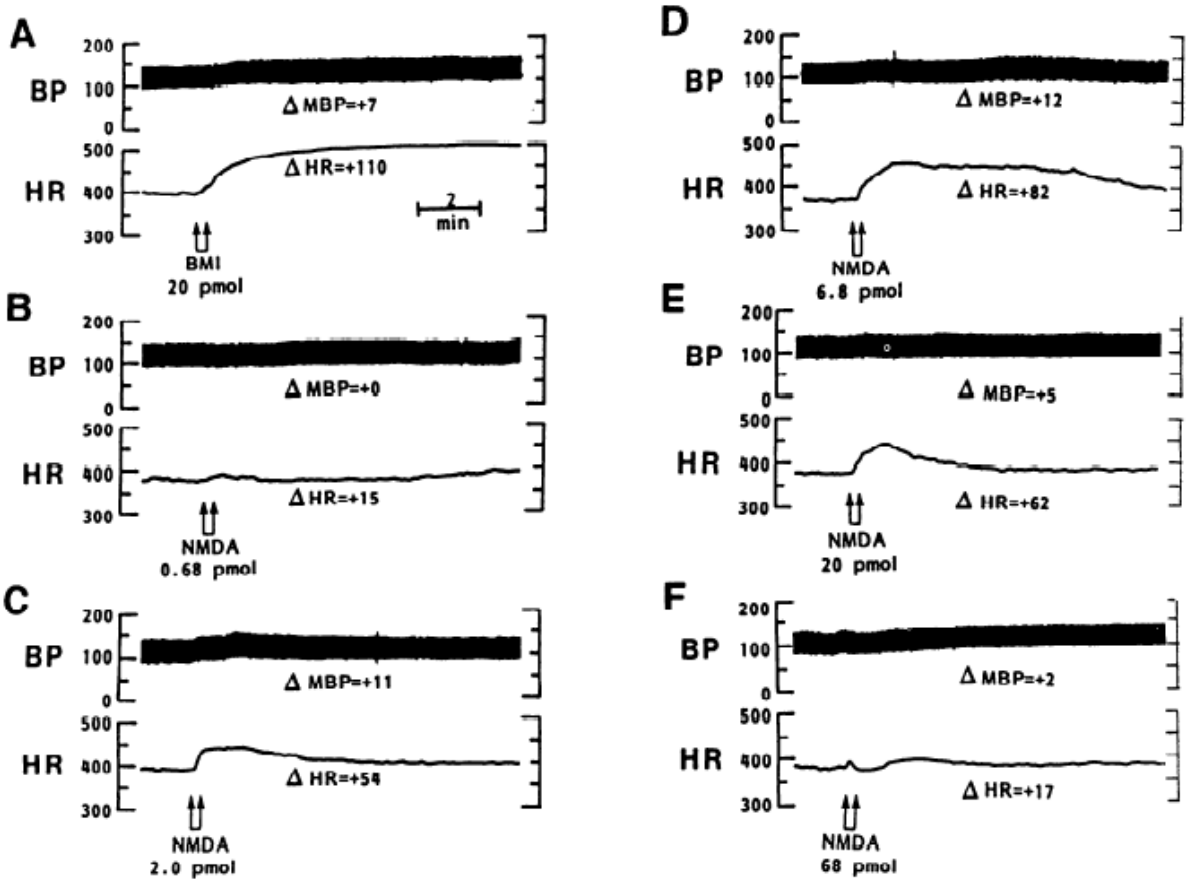
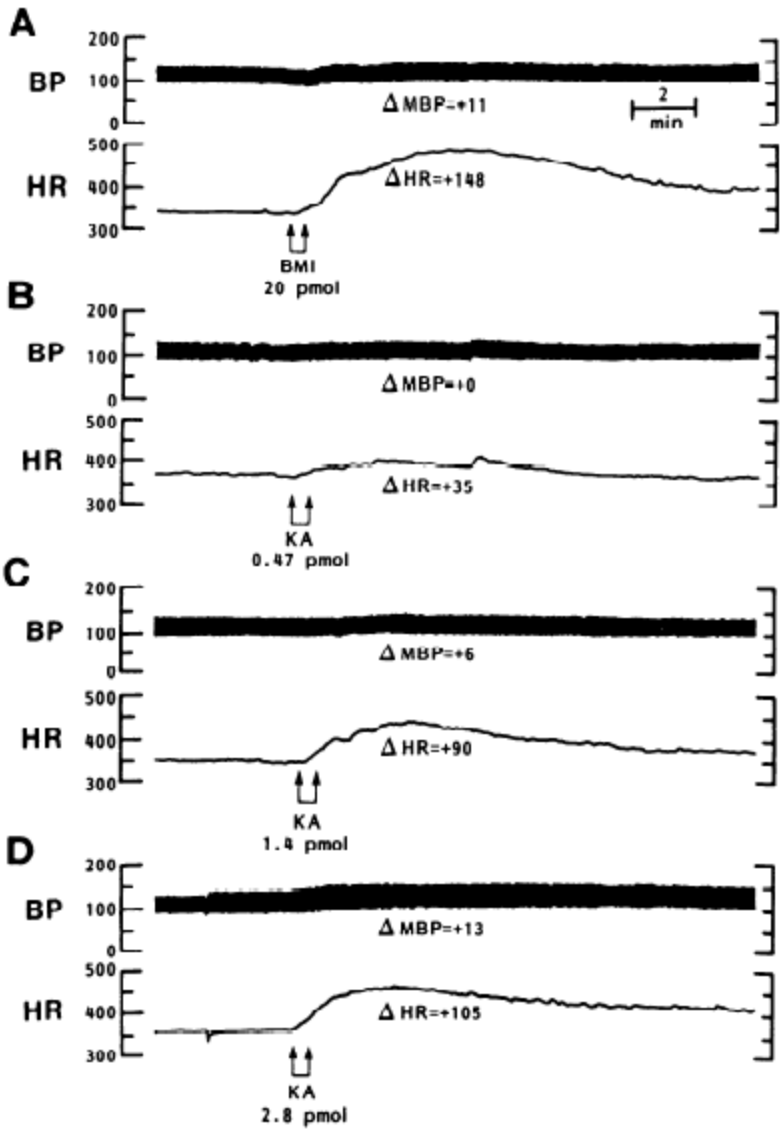


FIG. 1. Tracing of blood pressure (BP, mmHg) and heart rate (HR, beats/min) depicting effects of microinjection of bicuculline methiodide (RMI; A) and increasing doses of *N*-methyl-*D*-aspartic acid (NMDA; *B-F)* at same site in doromedial hypothalamus of a single urethan -anesthetized rat. Microinjection of 6.8 pmol NMDA 55 min after injection of 68 pmol NMDA (F) produced increases in HR and BP of 70 beats/min and 10 mmHg, respectively. Injections were made in 50 nl of saline and spaced 45-60 min apart. Maximum changes in BP and HR are indicated.



FIG;. 2. Tracing of blood pressure (BP, mm Hg) and heart rate (HR, beats/min) depicting effects of microinjection of bicuculline methiodide (BMI; *A*) and increasing doses of kainic acid (KA; *B-D)* at same site in dorsomedial hypothalamus of a single urethan-anesthetized rat. Injections were made in 50 nl of saline and spaced 45-75 min apart. Maximum changes in BP and HR are indicated.

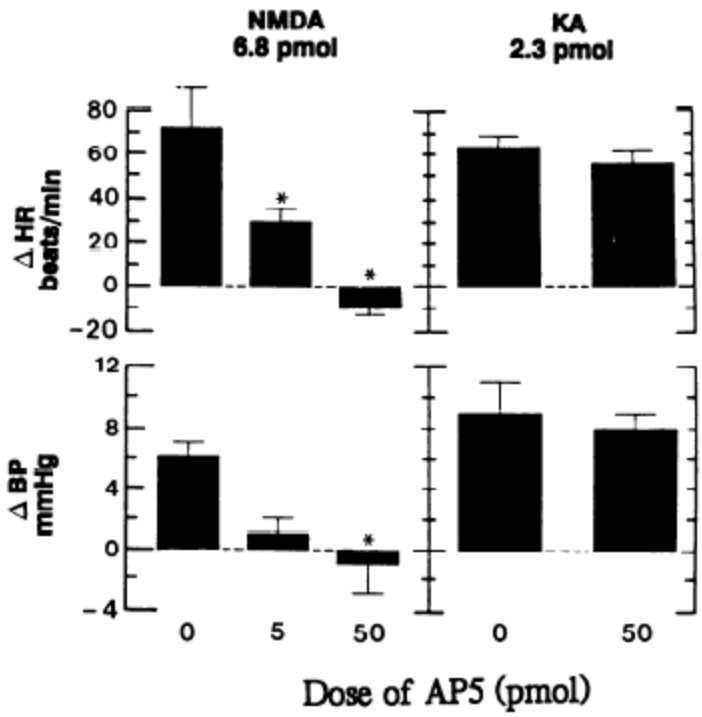


FIG. 3. Changes in heart rate (HR) and blood pressure (BP) caused by microinjection of *N*-methyl-D-aspartic acid (NMDA; *left)* and kainic acid (KA; right *)* alone or in the presence of 2-amino-5-phosphonopentanoic (AP5). AP5 was delivered in same solution as the agonists.

* Significant differences from NMDA alone ($P < 0.05$, by repeated ANOVA and Newman-Keuls, $n = 3$).

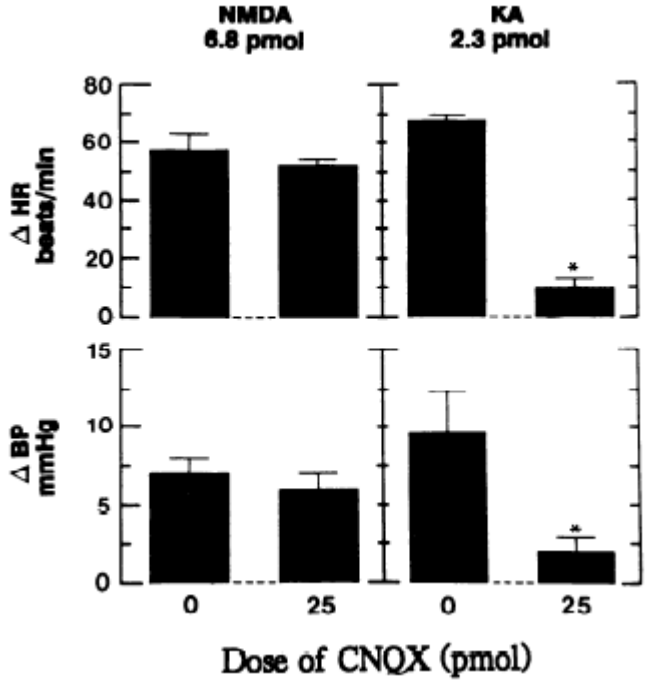


FIG. 4. Changes in heart rate and blood pressure caused by microinjection of NMDA (*left*) and KA (*right*) alone or in presence of 6-nitro- 7-cyanoquinoxaline-2,3-dione (CNQX). CNQX was delivered in same solution as agonists.* Significant differences from KA alone ($P < 0.05$, by paired *t* test, $n = 3$).

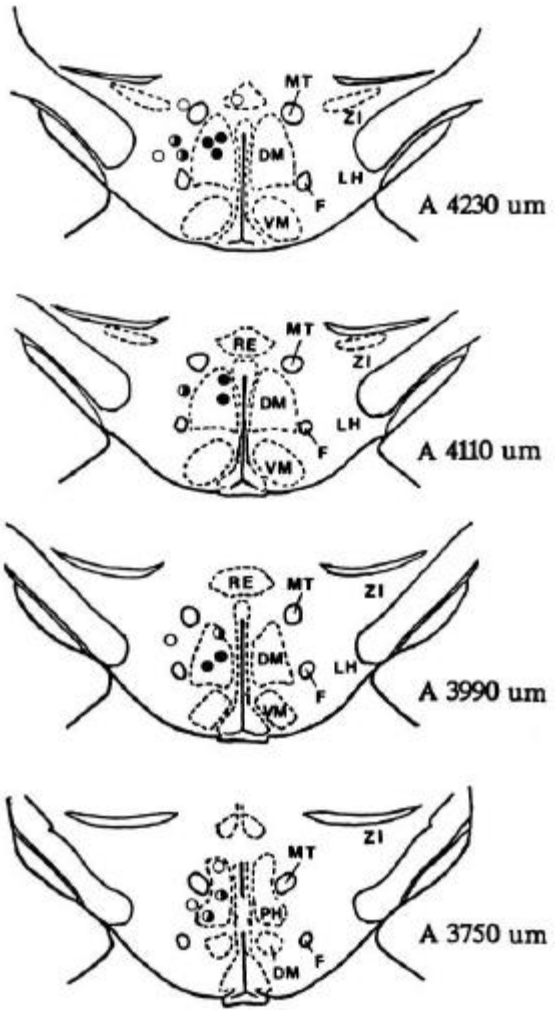


FIG. 5. Schematic coronal sections of rat brain adapted from Konig and Klippel (14). Effects on heart rate elicited by microinjections of 6.8pmol *N*-methyl-D-aspartic acid at each of 19 sites in 11 rats. Open circles: heart rate (HR) increased by <25 beats/min. Half-filled circles: HR increased by 25-49 beats/min. Filled circles: HR increased by ≥50 beats/min. DM, dorsomedial nucleus of the hypothalamus; DP, dorsal premammillary nucleus; F, fornix; LH, lateral hypothalamic area; MT, mammillothalamic tract; PH, posterior hypothalamus; RE, reuniens nucleus of the thalamus; VM, ventromedial hypothalamus; ZI, zona incerta; 3V, third ventricle.

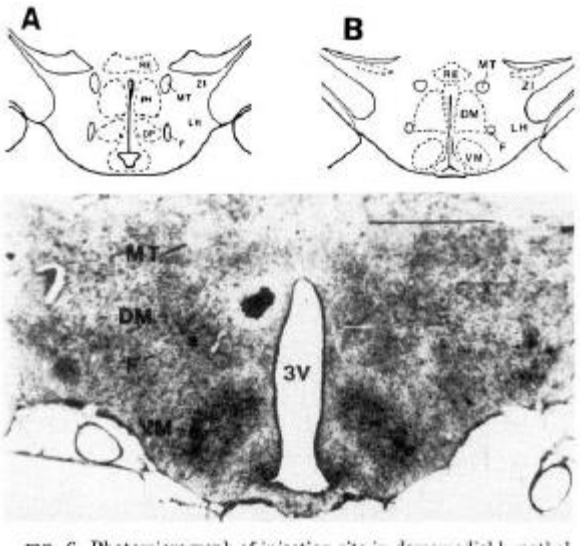


FIG. 6. Photomicrograph of injection site in dorsomedial hypothalamus at which 6.8pmol *N*-methyl-D-aspartic acid increased heart rate by 59 beats/min. Site was marked with 50 nl of india ink that was diluted 1:1 with saline. Bar, 1 mm. Schematic coronal sections of rat brain at comparable levels adapted from atlases of Pellegrino et al. (*A*) and Konig and Klippel (*B*). Abbreviations same as defined in Fig. 5 legend.