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Concurrent Hyperglycemic and Pressor Effects of Median Forebrain Bundle Stimulation in Rats[1] (41111)

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Abstract. Plasma glucose and blood pressure were concurrently increased by electrical stimulation of the median forebrain bundle (MFB) at different points in urethane-anesthetized rats. Neither response was reduced after bilateral adrenalectomy. Interruption of sympathetic tone by blocking α-adrenergic receptors with phentolamine, or by cervical spinal section, prevented hyperglycemia and the initial phase of the biphasic pressor response. In contrast, the secondary pressor phase was unaffected by these procedures but was abolished following hypophysectomy. Our results indicate that while hyperglycemia and the initial pressor phase were caused by sympathetic hyperactivity, the secondary pressor phase could be due to release of hormones like vasopressin from the neurohypophysis. Because hyperglycemic and pressor effects were elicited simultaneously from the MFB which interconnects various hypothalamic nuclei, it was considered possible that hypothalamic dysfunction could be a common denominator in elevating plasma glucose and blood pressure whenever diabetes and hypertension coexist.

Since hypothalamic stimulation elevates both blood sugar and blood pressure it seems conceivable that hypothalamic dysfunction might result in hypertension together with diabetes. However, changes in hypothalamic structure and function have been observed only in experimental hypertension (1–3), but not in diabetes. Furthermore, instead of being recorded concurrently, each response has usually been induced by stimulating separate hypothalamic sites in different animals: increases in blood pressure, pressor responses, from anterior, or posterior hypothalamic areas (4–6), and elevations in peripheral blood glucose levels, hyperglycemic responses, from the ventromedial hypothalamic region (7–9). In selecting brain areas from which both responses could be elicited the MFB seemed a logical choice since it connects hypothalamic nuclei not only to each other but also to other parts of the brain. Accordingly, our studies were designed to record simultaneous changes in arterial pressure and blood glucose occurring in the same animal during electrical stimulation of the MFB. Upon finding that both hyperglycemic and pressor effects could thus be elicited consistently even when the MFB was stimulated at different points, additional experiments were conducted to identify the mechanisms involved.

Methods. Female Sprague–Dawley rats, weighing 200–250 g, were purchased from either Charles River Breeding Laboratories (Wilmington, Mass.) or Hilltop Lab Animals Inc. (Scottdale, Pa.). In all experiments, the rats were anesthetized with urethane (0.1 g/100 g ip), and indwelling catheters were inserted through ventral incisions into a jugular vein and the lower abdominal aorta. Incisions were closed with sutures and the outer ends of both catheters were exteriorized so that the rat could be placed in a prone position in the stereotaxic apparatus. A concentric electrode with a proximal pole 0.5 mm in length and diameter, and a distal pole 0.5 mm long and 0.2 mm in diameter (NE-100 with a resistance

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of 50 Kohm in saline, by Rhodes Medical Instruments, Woodland Hills, Calif.), was then inserted into the MFB following the stereotaxic coordinates: rostrocaudal 6.0, mediolateral 2.0, and dorsoventral −3.0, using the atlas of Pellegrino *et al.* (10) as a reference.

During experiments, the outer end of the aortic catheter was connected to a pressure transducer (Statham P23Gb) to allow continuous recording of phasic blood pressure. For electrical stimulation, trains of 100 μA monophasic currents (pulse width 1 msec and frequency 100/sec with voltage set at 10 V) lasting for 3 min were delivered by connecting the implanted electrode to a square-wave stimulator (Grass S-48 with PSIU6 stimulus isolation unit). For determining plasma glucose, duplicate samples of arterial blood were collected three times in each experiment: (a) before stimulation, (b) at the end of 3 min of stimulation, and (c) 7 min after stopping stimulation. Using tuberculin syringes previously rinsed with heparin solution (1000 U/ml), 0.2-ml samples of blood were collected as needed and replaced with equal amounts of isotonic sodium chloride solution via the aortic catheter. Plasma aliquots from duplicate samples were then obtained for determination of glucose content (11).

Hypophysectomy through a parapharyngeal approach (12) or bilateral adrenalectomy through flank incisions, as well as the respective sham operations, were done under sodium pentobarbital anesthesia (40 mg/kg ip) 1 week before the rats were used for the stimulation study. Instead of regular drinking water, 5% glucose in isotonic sodium chloride for adrenalectomized rats, and 5% glucose in water for hypophysectomized rats, was given postoperatively. Hypophysectomized rats that on autopsy showed remnants of the anterior or posterior pituitary were not included in the final analysis. In some rats, the spinal cord was cut at the C1 level 30 min before MFB stimulation, and a tracheal cannula was inserted to allow connection to a positive-pressure respirator. For blocking α-adrenergic receptors, 4 mg/kg of phentolamine mesylate (Regitine, CIBA−Geigy), which reduced pressor responses to injected norepinephrine by about 90% in preliminary experiments, was injected through the jugular vein catheter.

After each experiment, a 2 mA direct current was passed through the implanted electrode for 5 sec to produce a small lesion at its tip. Through a thoracotomy, a perfusion needle was inserted into the ascending aorta via the left ventricle; 10% Formalin (in isotonic sodium chloride solution) was then perfused into the brain (13). Brains from nine randomly selected rats were stored in Formalin until sectioning; frozen sections 40 μm thick were cut transversely, stained with cresyl violet, and electrode sites were examined through a stereomicroscope were compared with an atlas for the rat brain (10). Lesions in six of nine rats were located in the MFB at the level of the paraventricular nucleus, while the rest were located slightly more rostrad and closer to the supraoptic nucleus (Fig. 1). Regardless of variations in electrode placement, results from all experiments were pooled and henceforth attributed to the MFB inasmuch as stimulation with 100-μA currents invariably increased both plasma glucose (>20 mg/100 ml) and blood pressure (>10 mm Hg) significantly.

Data expressed as averages ± SEM were analyzed using *t* tests for comparing means of dependent or independent samples (14) and differences at a 5% level ($P < 0.05$) or less were considered significant.

Results. *Effects of MFB stimulation in intact rats.* We used a current strength of 100 μA routinely for stimulation because in previous studies (4, 15) it always increased blood pressure moderately. Applied to the MFB for 3 min it produced biphasic elevations in aortic blood pressure which were invariably accompanied by significant increases in plasma glucose. Pressor responses usually consisted of an initial sharp rise lasting for about 30 sec, followed by a more sustained elevation throughout the remainder of the stimulation period (Fig. 2). Data summarized in Fig. 3 are from 26 rats in 23 of which plasma glucose was determined. Hyperglycemia was evident in all rats in which it was measured at the end of 3

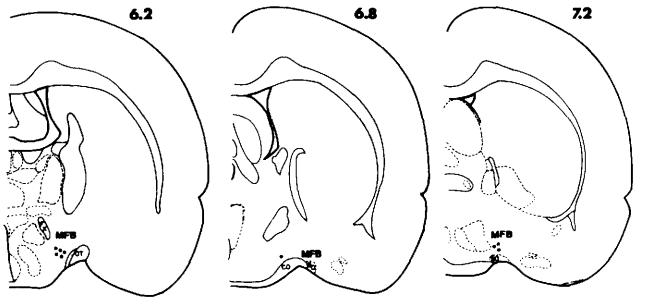

FIG. 1. Coronal section of rat brain at rostrocaudal coordinates 7.2 to 6.2 from the atlas by Pellegrino and Cushman (22). In addition to the MFB, surrounding structures include the fornix (FX), optic chiasm (CO), optic tract (OT), and supraoptic nucleus (SO). Black circles represent electrode sites found at each rostrocaudal coordinate: at 6.2 in 5 rats, at 6.8 in 1, and at 7.2 in 3.

min of stimulation, but was even more prominent 7 min after stimulation was discontinued.

Reproducibility of responses was determined by repeating stimulation at an interval of 30 min in four rats. Increases in mean aortic pressure produced by the first stimulation averaged 49 ± 7 mm Hg for the initial phase and 46 ± 2 mm Hg for the sustained phase. Corresponding pressor effects of the second stimulation were much smaller averaging 13 ± 6 mm Hg for the initial and 24 ± 7 mm Hg for the sustained phase. Hyperglycemia produced by the second stimulation (increases in plasma glucose were 44 ± 14 mg/100 ml after 3 min of stimulation and 67 ± 18 mg/100 ml 7 min later) seemed higher than that produced by the first stimulation (corresponding increases of 26 ± 9 and 43 ± 10 mg/100 ml, respectively); however, because of wide variations, differences between groups were not significant. Because these results show that neither pressor nor hyperglycemic responses remain constant, in all subsequent experiments each rat was stimulated only once and procedures designed to study underlying mechanisms were assessed by comparing group responses.

Does adrenalectomy alter responses to brain stimulation? The possibility that adrenomedullary catecholamines are released during stimulation was explored by comparing responses in eight adrenalectomized and eight sham-operated rats. Mean aortic pressure was initially lower in adrenalectomized (83 ± 3 mm Hg) than in sham-operated (109 ± 6 mm Hg) rats. Pressor responses to brain stimulation in adrenalectomized rats averaged 31 ± 4 mm Hg

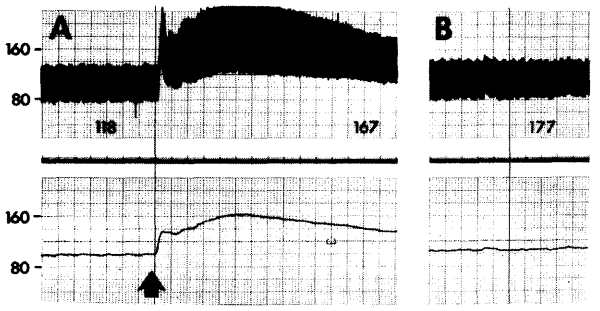

FIG. 2. Effects of hypothalamic stimulation in an intact rat anesthetized with urethane. Tracings of phasic (top) and mean (bottom) aortic pressure (mm Hg). Numbers below the phasic pressure tracing indicate plasma glucose (mg/100 ml), and the arrow indicates start of stimulation with 100 μA for 3 min. Panel A recorded before and during stimulation; panel B, 10 min later. Recorder speed 25 mm/min.

for the initial phase and 38 ± 5 mm Hg 3 min later; corresponding averages in sham-operated rats were 19 ± 3 and 32 ± 5 mm Hg, respectively. Before stimulation, plasma glucose levels averaging 123 ± 6 mg/100 ml in adrenalectomized rats were slightly lower than those of 140 ± 10 mg/100 ml in sham-operated ones; subsequent increases after 3 min of stimulation were almost the same (31 ± 4 mg/100 ml in adrenalectomized and 28 ± 6 mg/100 ml in sham operated), but those occurring 7 min later were lower in adrenalectomized (15 ± 5 mg/100 ml) than in sham-operated (37 ± 7 mg/100 ml) rats. As a whole these results indicate that while adrenal mechanisms might not contribute appreciably to the pressor effects of brain stimulation, they are responsible, at least in part for the post-stimulatory hyperglycemia.

Selective inhibition of responses to brain stimulation by α-adrenergic blockade or spinal section. To further test whether responses to brain stimulation were due to increased sympathetic neural activity, experiments were repeated following peripheral blockade of α-adrenergic receptors. Mean aortic pressure in 10 rats initially averaged 110 ± 3 mm Hg and then dropped to 84 ± 2 mm Hg after intravenous injection of phentolamine. From this new baseline, mean pressure during brain stimulation increased to 95 ± 2 mm Hg in the initial phase and to 113 ± 6 mm Hg at the end of 3 min (Fig. 4). Magnitude of the actual increase during the initial phase averaged 11 ± 1 mm Hg and was significantly smaller than that in untreated rats (see Fig. 3), but the sustained elevation was almost the same in both groups. By contrast, the baseline for plasma glucose of 132 ± 5 mg/100 ml after phentolamine treatment did not differ from that in untreated rats, and yet upon brain stimulation glucose levels instead of rising, fell to 114 ± 6 mg/100 ml after 3 min and to 113 ± 8 mg/100 ml 7 min later.

Following cervical section of the spinal cord in 11 other rats, baselines for mean aortic pressure averaged 67 ± 3 mm Hg and brain stimulation did not produce an initial pressor phase, but there was a secondary sustained elevation (Fig. 5) to 92 ± 7 mm Hg. The pressure increase of 25 ± 4 mm Hg during the secondary sustained elevation did not differ appreciably from that in intact rats (see below). Plasma glucose levels averaged 162 ± 9 mg/100 ml and were higher than any others seen previously; no significant changes occurred with brain stimulation (158 ± 8 mg/100 ml after 3 min and 161 ± 7 mg/100 ml 7 min later). These results, therefore, show that whereas hyperglycemic and initial pressor effects were inhibited by either α-adrenergic blockade or spinal section, the secondary sustained pressor effect was unaltered.

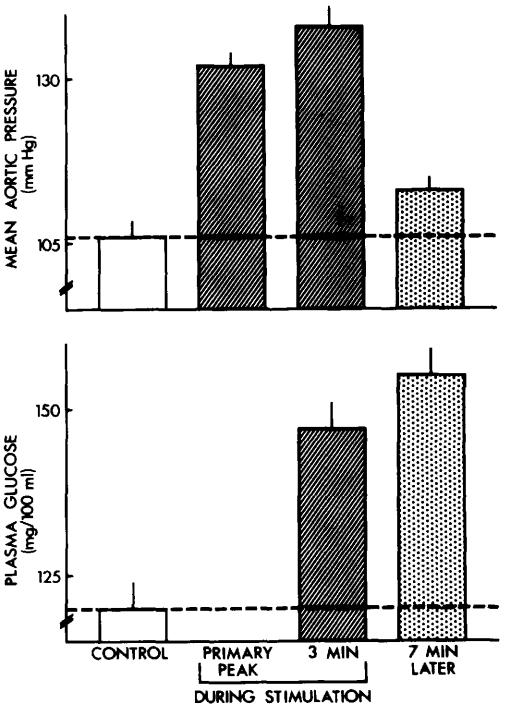

FIG. 3. Average changes in mean aortic pressure (n = 26) and plasma glucose (n = 23) produced by hypothalamic stimulation in intact urethane-anesthetized rats.

Absence of a sustained pressor response to brain stimulation in hypophysectomized rats. Because vasopressin has strong pressor activity and is synthesized in paraventricular and supraoptic nuclei of the hypothalamus (16), a release of endogenous vasopressin could account for the sustained pressor response. This possibility was studied by comparing blood pressure effects of brain stimulation in nine hypoph-

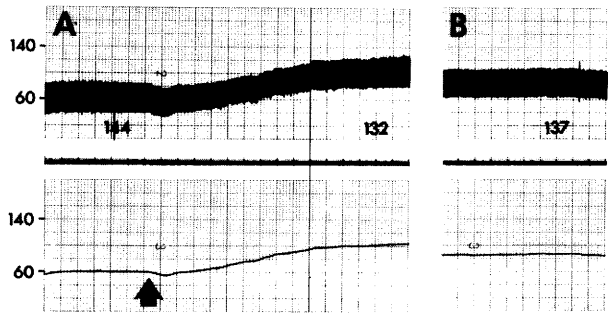

FIG. 4. Effects of hypothalamic stimulation in a rat pretreated with the α-adrenergic blocking drug, phentolamine. Rest of legend as in Fig. 2.

ysectomized rats with those in six sham-operated ones. The baseline for mean aortic pressure in hypophysectomized rats (95 ± 5 mm Hg) was significantly lower than that in sham-operated ones (121 ± 3 mm Hg), and the initial rise produced by stimulation was also less pronounced: mean pressure rose to 110 ± 6 mm Hg in hypophysectomized, and to 142 ± 4 mm Hg in sham-operated rats. A more striking difference was in the secondary sustained pressor phase which always occurred in sham-operated controls, but was either abolished (Fig. 6) or reversed in most hypophysectomized rats. After 3 min of stimulation, the mean pressure level in sham-operated rats of 139 ± 5 mm Hg was still significantly higher than the prestimulation level; by contrast, that in hypophysectomized rats averaged 85 ± 6 mm Hg and was lower than the prestimulation level. In seven of nine hypophysectomized rats, mean aortic pressures after 3 min of stimulation were actually lower than before stimulation began. Hence, by showing that the secondary pressor phase was abolished by hypophysectomy, these results suggest that the mechanism underlying the secondary response may involve release of pituitary hormones like vasopressin.

Discussion. Variability in electrode placement continues to be a bothersome source of error in studies employing stereotaxic methods even when differences in head alignment or in age or size of subjects are avoided. Although variability can be reduced by using the bregma as the skull landmark (instead of ear-bar-zero), in rats Slotnik and Brown (17) recently showed a median error, in locating brain points on a frontal plane, of 0.3 mm with a standard deviation of 0.38 mm. In our experiments, electrode positions (located in sections 7.2 to 6.2 mm anterior to the vertical zero plane on the rostrocaudal axis; see Fig. 1) certainly varied within the range they described, yet both hyperglycemic and pressor responses could still be elicited con-

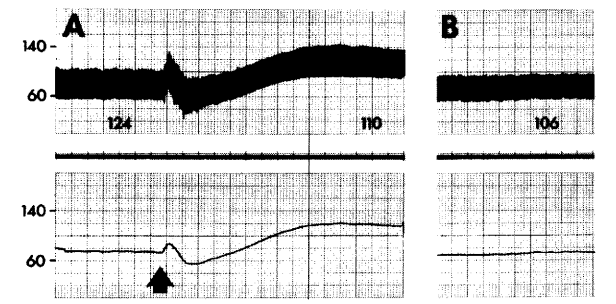

FIG. 5. Effects of hypothalamic stimulation following cervical spinal section. Rest of legend as in Fig. 2.

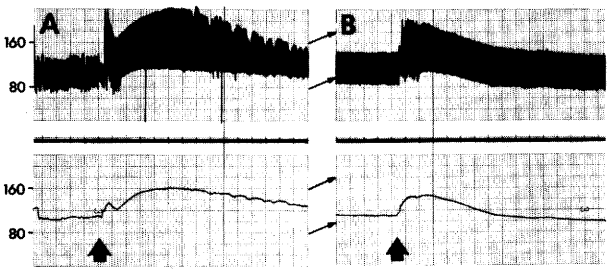

FIG. 6. Effects of hypothalamic stimulation on aortic pressure in a sham-operated (A) and a hypophysectomized (B) rat. Small arrows between panels indicate a change in vertical scales. Rest of legend as in Fig. 2.

sistently upon electrical stimulation with 100 μA for 3 min. Whether this was due to activation of a common pathway mediating both responses, or of two neighboring but separate pathways for each response, is conjectural. It is also possible that brain areas extending far beyond the immediate vicinity of the electrode tip may have been activated by current spread during the 3-min period of stimulation.

Nonetheless, our results indicate that at least two efferent mechanisms were involved: sympathetic hyperactivity for hyperglycemia and the initial pressor phase, and release of pituitary hormones (possibly vasopressin) for the secondary sustained pressor phase. Hyperglycemia and the initial pressor phase must have resulted mainly from sympathetic hyperactivity because they were both prevented either by blocking α-adrenergic receptors or by interrupting spinal pathways. In addition, adrenomedullary catecholamines may have elevated plasma glucose further after hypothalamic stimulation was discontinued since poststimulatory hyperglycemia was reduced in adrenalectomized rats. On the other hand, because the secondary sustained pressor phase was unaffected by these procedures but abolished by hypophysectomy, it probably involves release of pituitary hormones like vasopressin.

Inasmuch as responses to MFB stimulation closely resemble those elicited from various hypothalamic areas, it seems safe to assume that the effects we obtained were due, at least in part, to hypothalamic activation. Much of the hyperglycemia which can be induced by stimulating the ventromedial hypothalamus probably results from increased hepatic glycogenolysis initiated by sympathetic hyperactivity (18), but other mechanisms may also participate. Involvement of ACTH release has been implicated because ACTH can produce hyperglycemia, and the hyperglycemia induced by hypothalamic stimulation can be prevented by hypophysectomy (19). In addition, parasympathetic and adrenomedullary mechanisms have also been proposed because hyperglycemic responses to lateral hypothalamic stimulation can be inhibited by atropine pretreatment, vagotomy, or adrenal demedullation (20). Perhaps specific neurons exist for activating each mechanism separately such that the predominant mechanism would vary depending upon the brain areas stimulated.

Similarly, although biphasic pressor effects can also be elicited from the lateral (20, 21) or posterior hypothalamus (4, 5), underlying mechanisms may not always be the same. Initial pressor effects have been ascribed to sympathetic hyperactivity because they are inhibited by various types of autonomic blockade (e.g., of ganglia with pentolinium, of adrenergic neurons with guanethidine, or of α-adrenergic receptors with phentolamine). Secondary pressor effects have also been attributed to adrenomedullary catecholamines because they are abolished by adrenalectomy. Conflicting with this, however, Scherrer and Friedman (6) earlier found such pressor responses unaffected by adrenalectomy but reduced or abolished by lesions involving the infundibular hypophysis. While some discrepancies may have resulted from stimulation of different hypothalamic sites, the common possibility of vasopressin media-

tion implies that magnocellular neurons were being activated in all these studies. Although blood pressure could conceivably be elevated by other pituitary hormones like ACTH or somatostatin, there is considerable evidence implicating vasopressin. Stimulation of the supraoptic nucleus causes release of large amounts of endogenous vasopressin (22), and the possible contribution of this mechanism in experimental hypertension has recently been emphasized by the finding of increased plasma levels of vasopressin in rats with DOCA-salt (23), renal (24), or spontaneous (25) hypertension.

Interactions between experimental diabetes and hypertension apparently exist. Induction of alloxan diabetes increases pressor responsiveness to epinephrine, norepinephrine, and angiotensin (26), and elevates blood pressure in normotensive and spontaneously hypertensive rats (27). Conversely, hypertension accompanied by suppression of the renin–angiotensin system occurs in alloxan diabetic rats (28). And although most forms of experimental hypertension are not associated with hyperglycemia, in spontaneously hypertensive rats glucose tolerance is low (29) and caloric restriction lowers blood pressure (30). Notwithstanding uncertainties in interpretation, and recognizing that acute responses to brain stimulation may not be identical to chronic changes occurring in either diabetes or hypertension, our results allow us to speculate that some analogous disorder in brain function could be involved in the etiology of both diseases.

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