Depression and Enhancement of Baroreceptor Pressor Response in Cats after Intracerebroventricular Injection of Noradrenergic Blocking Agents

Dependence on Supracollicular Areas of the Brain

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SUMMARY The α-adrenergic blocking drugs, phentolamine and Hydergine, both act centrally at different sites to depress and enhance the pressor and sympathetic nerve response to decreased baroreceptor afferent input in anesthetized cats. Depression of the rise in blood pressure and sympathetic nerve discharge during bilateral carotid occlusion (BCO) followed injection of the agents into the 4th cerebral ventricle when the brain was intact but not when connections were interrupted at the midcollicular level by transaction or lesion. Enhancement of responses occurred when drug distribution was confined to the brain rostral to the midcollicular level via injection into the 3rd cerebral ventricle with the cerebral aqueduct cannulated.

IN 1930, WRIGHT1 reported that the ergot-type α-adrenergic blocking agent, ergotamine, when administered intravenously to cats blocked the bilateral carotid occlusion (BCO) pressor response in doses that did not produce peripheral noradrenergic blockade. Depression of the BCO response was attributed to a drug action in the central nervous system. Subsequently, other ergot derivatives with peripheral noradrenergic blocking activity were reported to act centrally to depress the BCO pressor response.2 3 Wright1 found that he could demonstrate this central effect in cats with the brain intact and anesthetized with chloralose, but not in unanesthetized cats decerebrated at the midcollicular level. He suggested that the presence of the anesthetic was required to demonstrate the central blocking actions. An alternate explanation is that the drug blocks the BCO pressor response by acting on neural pathways which connect supracollicular areas of the brain with the caudal brainstem. Thus the action of the drug might be prevented by decerebration because the intervention interrupts the pathways surgically. If this is the case, it follows that the activity normally present in these pathways when the brain is intact is an important determinant of the amplitude of the carotid occlusion pressor response.

Both agents decreased resting blood pressure and Hydergine decreased heart rate in intact and decerebrate preparations but not in 3rd ventricle-cerebral aqueduct experiments. We found that pretreatment with the noradrenergic precursor, l-dopa consistently prevented depression by phentolamine but was less effective against Hydergine. The results indicate that mechanisms which enhance and suppress the baroreceptor pressor response are normally operative in anesthetized cats and, furthermore, that neural pathways mediating the effects are ones connecting the caudal brainstem with supracollicular levels of the brain. It is further suggested that the pathways may be noradrenergic.

In the present experiments the BCO response was tested after injecting the ergot-type blocking agent, Hydergine, into the 4th cerebral ventricle of cats in which the brain was intact or transected at the midcollicular level. To further localize the site for depression of the response, injection and drainage cannulas were placed in the 3rd cerebral ventricle and cerebral aqueduct so as to confine drug distribution to the brain rostral to the midcollicular level. To suggest whether results might be peculiar to ergot compounds, similar experiments were performed with a structurally different adrenergic blocking agent, phentolamine. This agent was reported to act centrally to depress the BCO pressor response following intracerebroventricular injection in dogs.4

Methods

Experiments were performed on 40 cats anesthetized with chloralose, 55 mg/kg, and urethane, 250 mg/kg, administered intraperitoneally. The trachea and a femoral artery and vein were cannulated, the cervical vagosympathetic nerves were sectioned, and the common carotid arteries prepared for bilateral occlusion (BCO). The duration of BCO was 30-45 seconds in the different experiments. Arterial blood pressure was measured with a Statham P23AC transducer and heart rate with a tachograph triggered by the arterial pressure pulse.

To minimize the contribution of carotid chemoreceptor stimulation to the carotid occlusion pressor response, cats were paralyzed with gallamine (10 mg/kg, iv) and artificially ventilated with 40% O₂ in N₂. The amplitude of the BCO pressor response in individual cats did not change when inspired O₂ concentration was increased from 40% O₂ to 100% O₂, therefore the lower concentration was used.
INJECTION INTO 4TH CEREBRAL VENTRICLE

The cat's head was fixed in a stereotaxic instrument, and a cannula made from a 22-gauge spinal needle was placed in the 4th ventricle through a hole in the occipital bone. A caudal insertion angle of 36° was used and the cannula tip was placed 2–4 mm rostral to the obex in the midline. Location of the cannula tip was confirmed visually at the end of each experiment. Drugs were injected in a volume of 0.1–0.2 ml of Ringer-Locke solution.

DECEREBRATION

Surgical decerebration was performed by transection which interrupted the brain dorsally at the midcollicular level and ventrally at the rostral termination of the pontine gray. The brain rostral to this level was aspirated and bleeding was controlled by occluding the basilar artery with a MacKenzie clip and by packing the sella turcica with oxidized cellulose (Oxycel, Parke, Davis). Decerebration by lesion was accomplished along the same frontal plane as surgical transection with a Grass high frequency lesion-maker and bipolar concentric electrodes. Lesions were placed at 1-mm intervals along tracts 1, 2, and 3 mm from the midline bilaterally. Pupils were dilated bilaterally after placing the lesions. At the end of the experiment the brain was perfused with 10% formalin through a cannula made from a 22-gauge spinal needle was placed in the ascending aortic arch and later sectioned to confirm the extent of the lesions.

INJECTION INTO 3RD CEREBRAL VENTRICLE WITH THE CEREBRAL AQUEDUCT CANNULATED

The injection cannula was introduced into the brain at A, 12.0 in the midline and advanced until the tip entered the 3rd cerebral ventricle. A portion of the occipital bone was removed and the cerebellum overlying the 4th cerebral ventricle was elevated so that the opening of the cerebral aqueduct into the 4th ventricle was visualized. A polyethylene tube was inserted into the aqueductal opening and advanced 2–3 mm, until it was securely lodged in the aqueduct. The tube carried all fluid draining from the cerebral aqueduct to the exterior, thus bypassing the 4th ventricle. Tightness of fit was considered satisfactory when a volume of saline injected into the 3rd ventricle could be collected without loss from the aqueductal cannula. At the end of the experiment, 0.1–0.2 ml of Evans blue was injected into the 3rd ventricle-aqueduct system and the distribution was checked visually. There was no evidence of coloration in the 4th ventricle. Dye was detected in the proximal portion of the horns of the lateral ventricles. Drugs were injected in a volume of 0.05 ml into the 3rd ventricle.

NERVE RECORDING

The lowest three ribs were removed on the right side and the right greater splanchnic nerve was freed from surrounding tissue, desheathed, and placed on bipolar silver-silver chloride electrodes. A mineral oil pool was formed using Parafilm and the nerve was covered with cotton soaked in mineral oil. The opening in the chest was covered with several layers of Parafilm. Temperature in the pool was maintained between 36°C and 37°C by means of an infrared heating lamp regulated by a rectal thermistor probe. Potentials were conventionally amplified, displayed on an oscilloscope, and introduced into an audio amplifier. Action potentials also triggered a pulse height selector which produced a square wave of constant output and duration. Action potentials and the output of the selector were both continuously monitored on an oscilloscope, and the triggering level of the selector was set to exclude noise. Any single potentials or groups of summated potentials (bursts) above the noise level triggered the selector. A rate meter circuit which received its input from the pulse height selector provided a signal whose amplitude varied directly with the frequency of action potentials but did not give the absolute impulse frequency. The time constants of the rate meter and ink-writing recorder were adjusted so that variations in sympathetic impulse frequency related to ventilation and to spontaneous fluctuations in blood pressure were reflected accurately in the recorder trace. The recording system was calibrated by introducing 1-msec pulses at known frequencies into the pulse height selector.

Initially, in some experiments there was a relatively small increase in impulse frequency recorded from the whole, desheathed splanchnic nerve during BCO. However, after splitting the nerve, it always was possible to find a subdivision containing fibers whose frequency of discharge increased more markedly during BCO.

DRUGS USED

For these experiments we used a mixture of dihydrogenated ergocornine, ergocristine, and ergotryptine methanesulfonates (Hydergine), phentolamine sulfonate (Regitine), norepinephrine bitartrate (Levophed), phenylephrine hydrochloride (Neo-Synephrine), α-chloralose (Aldrich), ethyl carbamate (urethane, Aldrich), and gallamine triethiodide (Flaxedil).

RESULTS

CATS WITH INTACT BRAIN

Phentolamine or Hydergine was injected into the 4th cerebral ventricle and the dose was increased until the pressor response to BCO was reduced to approximately 50% of the control value (Table 1). Control intraventricular injection of Ringer-Locke solution with a pH corresponding to that of the phentolamine or Hydergine solutions did not alter cardiovascular variables or sympathetic nerve discharge. For phentolamine the total dose required was 0.3–1.0 mg, and for Hydergine the total dose was 90–120 μg. Norepinephrine (NE), 0.5–1.0 μg/kg or phenylephrine (PE), 5–10 μg/kg was injected intravenously (iv) periodically throughout the experiments to determine whether there was peripheral noradrenergic blockade during depression of the BCO response. As shown in Table 1, the pressor response to iv NE or PE was not decreased during depression of the BCO response.

Resting mean arterial pressure (MAP) decreased after injection of phentolamine or Hydergine but usually remained above 100 mm Hg at the time of maximum depression of BCO (Table 1). Also, there was marked bradycardia after Hydergine but no significant change in heart rate (HR) after phentolamine (Table 1 and Figs. 1 and 2).
The time course of depression and recovery of the BCO response after phentolamine and Hydergine is shown in Figure 3. The response began to decrease within 5 minutes after injection into the 4th cerebral ventricle, and depression was maximum 10–20 minutes after injection. After phentolamine, recovery was almost complete within 90 minutes; however, there was only partial recovery at this time after Hydergine. In two experiments with Hydergine, the BCO response was tested for an additional 60 minutes and no further recovery was observed.

Preganglionic sympathetic impulses were recorded from the splanchnic nerve to confirm that depression of the BCO pressor response was due to a central drug action. Recordings were made in four of the cats with intact brains included in the phentolamine and Hydergine groups (Table 1), and portions of original records are shown in Figures 1 and 2.

![Figure 1](https://example.com/image1)

**Figure 1** Depression of bilateral carotid occlusion (BCO) pressor and splanchnic nerve responses following injection of phentolamine into the 4th cerebral ventricle of a cat with an intact brain. Both panels from same experiment. Top panel (4), continuous record of control responses: (1) BCO, (2) norepinephrine (NE), iv; (3) BCO. Bottom panel (8), continuous record starting 6 minutes after injection of phentolamine (1 mg total dose) into 4th cerebral ventricle: (1) BCO, (2) BCO, (3) NE, iv.
the group with intact brains. Also, the marked bradycardic effect of Hydergine still was present in the decerebrate preparations. After administration of phentolamine the changes in HR were variable, and no significant trend was apparent.

Four of the 10 preparations tested with the blocking agents were decerebrated by high frequency lesions which preserved the ventricular system. This was done to confirm that the absence of BCO blockade observed first in surgically transected preparations was not due to loss of part of the injected dose from the transected cerebral aqueduct. This possibility appeared unlikely with the injection volume of 0.1–0.2 ml normally used, since the same volume of Evans blue injected into the 4th cerebral ventricle produced no apparent coloration of the transected end of the cerebral aqueduct. However, coloration was sometimes apparent when larger volumes (0.4–0.5 ml) of dye were injected.

3RD VENTRICLE-CEREBRAL AQUEDUCT INJECTION

In these six experiments the drug distribution was confined to the brain rostral to the cerebral aqueduct. The agents did not enter the 4th cerebral ventricle and consequently did not exert an action via the cerebrospinal fluid on brainstem structures caudal to the midcollicular level. Under these conditions the amplitude of the BCO pressor response increased after phentolamine (1.0 mg) and Hydergine (200 μg) (Table 2). Segments of an original record corresponding to that of L-dopa solutions caused slight hypertension and increased sympathetic discharge during injection, but the effect was transient. L-dopa itself did not significantly change the amplitude of the BCO pressor response. Thus, in the four cats that subsequently received phentolamine (Fig. 3), the BCO pressor response was 65 ± 6 mm Hg before and 70 ± 8 mm Hg 1 hour after L-dopa. However, as illustrated in this figure, pretreatment with to the caudal brainstem (compare Tables 1 and 2). The marked bradycardic effect of Hydergine observed in intact and decerebrate groups was absent in the 3rd ventricle-cerebral aqueduct preparations, and neither phentolamine nor Hydergine decreased resting MAP in these preparations. Nerve recordings indicated that resting sympathetic discharge did not change after phentolamine or Hydergine but that the increase in frequency in response to BCO was greater (Figs. 4 and 5). These observations are consistent with the view that the drug effects are due to an action in the central nervous system.

EFFECTS OF L-DOPA AND NORADRENERGIC AGONISTS ON BCO BLOCKADE

In four preliminary experiments NE or PE was injected into the 4th cerebral ventricle of cats with intact brains to see whether the effect of the blocking agents could be prevented or reversed. However, the effects of NE and PE alone on blood pressure and sympathetic discharge suggested a complex interaction of central and peripheral drug effects which was difficult to interpret. A major problem was that the amplitude of the BCO pressor response often became highly variable after intracerebroventricular injection of NE or PE. For these reasons, the noradrenergic precursor, L-dopa, was tested in subsequent experiments. Cats were pretreated with L-dopa (total dose = 1 mg) injected into the 4th cerebral ventricle 1 hour before the blocking agent was administered. Injection of Ringer-Locke solution with a pH corresponding to that of L-dopa solutions caused slight hypertension and increased sympathetic discharge during injection, but the effect was transient. L-dopa itself did not significantly change the amplitude of the BCO pressor response. Thus, in the four cats that subsequently received phentolamine (Fig. 3), the BCO pressor response was 65 ± 6 mm Hg before and 70 ± 8 mm Hg 1 hour after L-dopa. However, as illustrated in this figure, pretreatment with
For explanations, see footnotes to Table 1.

L-dopa did consistently prevent depression of the BCO pressor response by phentolamine (total dose = 1.5 mg). L-Dopa was less effective in preventing depression by Hydergine. In two of four experiments pretreatment did prevent depression of the BCO response and bradycardia after Hydergine (total dose = 120 μg). In a third experiment bradycardia was prevented but depression of the BCO response was not; and in the fourth experiment pretreatment with L-dopa prevented neither depression of the BCO response nor bradycardia.

Administration of L-dopa after depression of the BCO response by phentolamine (total dose = 1 mg) or Hydergine (120 μg) did not reverse the depression in four experiments even though a total of 3 mg was injected into the 4th cerebral ventricle.

Discussion

The increase in blood pressure and sympathetic discharge elicited by BCO in the present experiments is a reflex response to reduce baroreceptor afferent input. The contribution of chemoreceptor afferent excitation to the BCO response was prevented by ventilating cats with 40% O₂. Even in cats breathing room air, the contribution of chemoreceptor stimulation to the BCO pressor response is highly variable and impulse frequency in fact does not increase in many carotid chemoreceptor afferents during occlusion under these conditions. It previously has been shown that the carotid chemoreflex pressor response is not decreased by ergot-type noradrenergic blocking agents which act centrally to depress the BCO pressor response.

Hydergine and phentolamine depress the BCO response when they are injected into the 4th cerebral ventricle of cats with an intact brain. Since the response is enhanced after injection into the 3rd ventricle-cerebral aqueduct preparations, depression is attributed to a drug action in the brainstem caudal to the cerebral aqueduct. Neither agent depressed the BCO response in decerebrated preparations. This observation indicates that neural pathways connecting the caudal brainstem with supracollicular areas of the brain must be intact for the drugs to cause depression. Whether the pathways affected by Hydergine and phentolamine ascend to or descend from supracollicular levels, we cannot say. However, to account for the drug-induced depression
we suggest that functionally these pathways were enhancing the BCO pressor response at the time that the blocking agents were injected into the 4th cerebral ventricle. In contrast, injection of the agents into the 3rd ventricle-cerebral aqueduct preparations enhanced the BCO response. This result indicates that there is also a supracollicular site at which the drugs act and suggests that, functionally, this site is within a pathway that was suppressing the BCO pressor response at the time of drug administration.

Our interpretation of the pharmacological experiments is consistent with reports that electrical stimulation of supracollicular sites can enhance or suppress baroreceptor reflexes and that baroreceptor afferent input affects the activity of neurons in supracollicular areas of the brain. Inconsistent with this interpretation is the finding that supracollicular influences, at least on the amplitude of the BCO response, are not revealed by transection experiments. In the present and in previous studies, the amplitude of the response did not change after midcollicular transection. These observations, however, establish only that the essential afferent and efferent pathways for the response are intact in the caudal brainstem, and this conclusion does not preclude the possibility that supracollicular areas influence the amplitude of the response when the brain is intact. Indeed, the only situation in which transection must invariably affect the amplitude of the response is when it does ablate part of the essential reflex pathway. It may also be the case that the contribution of higher centers is demonstrated in pharmacological and stimulation experiments because neural pathways can be affected selectively by these methods. The action of the blocking drugs in our experiments depended on the areas of the brain exposed to the agents; and in previous stimulation experiments, the occurrence of suppression or enhancement of baroreceptor reflexes depended on the site of stimulation. In transection experiments, on the other hand, the interruption of neural pathways is nonselective.

From our results, further suggestions can be made about the caudal brainstem site at which phentolamine and Hydergine act to depress the BCO response. First, the site is not within the central baroreceptor afferent pathway. Pharmacological blockade here should increase resting blood pressure in the same manner as surgical interruption of baroreceptor afferents, and we observed a decrease in MAP and sympathetic discharge coincident with depression of the BCO response. It is more likely, therefore, that the agents act within a central efferent pathway to attenuate sympathetic activation when baroreceptor afferent input is decreased. Second, within the efferent pathway, direct depression of preganglionic sympathetic or medullary bulbo-spinal neurons may be excluded. If the drugs act directly on these structures, then depression of the BCO response should be observed in decerebrate preparations; and this is not the case. Depression is most readily explained by a more indirect action possibly at an interneuronal site within a pathway descending from supracollicular levels of the brain. Functionally this pathway would facilitate the activation of preganglionic sympathetic neurons that occurs when baroreceptor afferent input is decreased.

Although supracollicular pathways appear to be involved in depression of the BCO response by Hydergine, they do not mediate the decrease in resting heart rate observed with this agent. Bradycardia occurred in decerebrate and intact preparations but not in the 3rd ventricle-cerebral aqueduct experiments. Consequently, this drug effect can be attributed solely to an action in the brainstem caudal to the cerebral aqueduct. Since all preparations were vagotomized, Hydergine must act in the caudal brainstem to decrease activity in sympathetic nerve to the heart.

Hydergine and phentolamine are structurally unrelated compounds that have in common the ability to produce peripheral noradrenergic blockade. In the present experiments, pretreatment with the noradrenergic precursor, L-dopa, consistently prevented central depression of the BCO response by phentolamine but did not effect the response itself. This evidence, though indirect, supports the view that the action of phentolamine was due to central noradrenergic blockade. In a previous study of ergot derivatives, including Hydergine, it was concluded that central depression of the BCO response was not correlated with peripheral α-adrenergic blocking potency. However, central noradrenergic receptors may differ from those in the periphery, and the study did not test whether depression could be prevented or reversed pharmacologically. Our results suggest that pretreatment with L-dopa can prevent depression of the BCO response by Hydergine, although results were not as consistent as those obtained with phentolamine. Since Hydergine produces a more persistent noradrenergic blockade than phentolamine, it may be more difficult to prevent the block competitively by pretreatment with the noradrenergic precursor.

If the central effects of phentolamine and Hydergine are due to noradrenergic blockade, then the possibility arises that activation of different central noradrenergic systems can suppress and enhance the preganglionic sympathetic response to decreased baroreceptor afferent input. It has been reported that two other α-adrenergic blocking agents, tolazoline and piperoxan, enhance the pressor response to electrical stimulation of the hypothalamus when they act on the caudal brainstem (medulla) and suppress the response when they act on supracollicular structures (hypothalamus).

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Effect of Quinidine and Temperature on Sodium Uptake and Contraction Frequency of Cultured Rat Myocardial Cells

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SUMMARY The effects of quinidine and temperature on Na influx and contraction frequency of synchronously contracting rat myocardial cells in monolayer cultures were studied. Quinidine (10^{-4} M to 10^{-2} M) produced a prompt reduction in Na influx, maximum after 30 seconds of exposure, and dose-dependent along a sigmoid log dose-response curve. At 37°C, Na influx (µmol/10^9 cells per sec) decreased from 30.19 to 24.70 (P < 0.001) and 10.49 (P < 0.001) on exposure to quinidine, 10^{-6} and 10^{-4} M, respectively. Simultaneously, the contraction frequency decreased from a control of 120/min to 105/min and 48/min with 10^{-4} M and 5 x 10^{-4} M quinidine. At higher concentrations spontaneous contractions ceased. The effects on Na influx and contraction were reversible by washing the cells free of the drug (30 seconds). A temperature-dependent decrease in the Na influx between 37°C and 22°C also induced a decrease in contraction frequency. Between 25°C and 35°C the Q_{10} values for Na influx and contraction frequency were 2.41 and 2.44, respectively. Under all conditions tested there was a constant linear relationship (r = 0.98) between Na influx and contraction frequency for all values of Na influx greater than 11.82 µmol/10^9 cells per sec. Na influx and contraction frequency were insensitive to tetrodotoxin (10^{-4} g/ml) but very sensitive to verapamil and to changes in extracellular Na. Quinidine affected only the verapamil-sensitive Na influx. The results indicate a close relationship between verapamil-sensitive inward Na movement and automaticity in these cells and demonstrate that the quinidine-induced changes in automaticity are closely linked to the effect on Na influx.

ELECTROPHYSIOLOGICAL studies have shown that quinidine decreases automaticity of cardiac tissue, primarily decreasing the rate of depolarization during phase 4 of the cardiac action potential. Several studies also have shown a marked effect of quinidine on myocardial sodium exchange. In rabbit aatria quinidine reduced the passive movement of sodium into the cell, finding similar to that reported for cat papillary muscle. McCull used cultured human cells and demonstrated a dose-dependent effect of quinidine on passive sodium influx and membrane permeability to that ion. Studies such as these have prompted the conclusion that the action of quinidine and quinidine-like agents is related, in some way, to an alteration in membrane permeability to sodium and potassium, but direct evidence, especially with regard to sodium, still is lacking.

The present study was performed to explore the relationship of the effects of quinidine and temperature on Na uptake to their effect on automaticity in cultured rat myocardial cells. These cells, grown until a confluent monolayer is formed, show spontaneous, synchronous contractions in vitro that are representative of intrinsic automaticity. Many of the electrophysiological features of these cells indicate that their action potentials are slow channel-dependent and that they are similar to cardiac pacemaker tissue in various situations. As has been pointed out, these cells also possess the advantages provided by other cultured cells for ion flux studies in that, even when grown as a monolayer, cultured cells may be quickly and completely removed from the extracellular fluid, making possible the detection of small changes in

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