Effects of Cyclopropane, Halothane and Procaine on the Vasomotor "Center" of the Dog

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In a series of vagotomized, decerebrated dogs, the medulla oblongata was explored for maximal "pressor" and "depressor" responses to electrical stimulation. The stimuli were delivered from a concentric needle electrode which contained a hollow core. After the most responsive areas had been located, the effects of cyclopropane, halothane, and procaine were compared by injecting through the needle a small standard volume of saline solution which contained one of these anesthetics. All three agents caused reversible depression of the responses from both pressor and depressor areas. However, the effects of halothane and procaine were equal in both areas, while cyclopropane had a disproportionately small effect upon pressor representations. When halothane and cyclopropane were compared in equinarcotic concentrations, halothane was found to depress the pressor response to twice the extent that cyclopropane did. The importance of these results in explaining the hemodynamic effects of the anesthetics is discussed.

We have suggested[1,2] that cyclopropane causes arterial hypertension by means of actions exerted within the medulla oblongata. The mode of action proposed by us involves a selective inhibition by cyclopropane of the vasodepressor neurones in the hindbrain. The evidence for this view is indirect; it is based upon the principle of exclusion because demonstration of a direct action has not been practicable.

Although the means of demonstrating these actions by direct observation do not yet exist, new methods of study are available, and have been utilized in the present study. The results suggest, again, that cyclopropane is unusual in that it does inhibit medullary vasodepressor representations to a greater extent than pressor areas. In contrast, two other anesthetics which were examined had equal effects on pressor and depressor representations.

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Methods

Anesthesia was induced in unpremedicated, fasting, mongrel dogs (10–15 kg.) with halothane, nitrous oxide, and oxygen, utilizing carbon dioxide absorption and intermittent positive pressure breathing. After bilateral vagotomy and fixation of the head in a stereotaxic instrument, the hindbrain was exposed by partial removal of the temporal lobe, and midcollicular decerebration was accomplished with a hot wire under direct vision. The anesthetics were discontinued two hours or more before the study began. Respirations were controlled with hyperventilation at constant minute volume during both the surgical preparation and the period of study. The brain stem was exposed by separating the posterior neck muscles in the midline and dissecting them from the occiput. The mid-portion of the occipital bone was removed and the dura incised so that the medulla oblongata and the posterior cerebellum were exposed. The cerebellum was lifted by blunt dissection or partially removed by suction and cautery to expose completely the caudal part of the floor of the fourth ventricle.

Vasopressor and vasodepressor areas of the medulla were explored for maximal responses of arterial pressure with a shielded bipolar needle electrode mounted on a stereotaxic instrument equipped with a drive unit. The electrode had an external diameter of 0.7 mm., a 0.3 mm. tip separation, and a hollow core of 0.2 mm. diameter. A 50 μl. syringe was attached to its proximal end. The medulla was

explored by plunging the electrode to a depth of 8 mm., then withdrawing it in 0.3 mm. steps. Exploration was terminated 1 mm. from the brain surface. The surgical preparation was electrically isolated by using an isolation transformer and wood supporting table. Medullary stimulation was achieved using a frequency of 100 or 200 c.p.s., a pulse duration of 1 millisecond, a train of 10 seconds duration, and a constant peak current flow which ranged in individual experiments from 0.02 to 0.15 ma. Current flow was estimated with an oscilloscope by measuring the voltage drop across a 100 ohm resistor placed in series with a ground lead on an isolation transformer. The central element was uniformly negative with respect to the outer casing. Stimuli were applied to the medulla at 1 minute intervals for exploration and 2 or 5 minute intervals for control and experimental observations. Two identical electrodes were used throughout the experiments and the same amperage was produced (on the average) whether the area stimulated was "pressor" or "depressor."

Substances for study were dissolved in or equilibrated for 15 minutes with normal saline which was subsequently injected into the brain through the needle electrode used for stimulation. The duration of each injection was 20 seconds. Stimuli following injection were given at 1, 3, and 5 minutes, and at 5 minute intervals thereafter. The amperage applied was adjusted to equal that recorded during the control responses. When, following an injection, the magnitude of the response was reduced, it was required that recovery to within 25 per cent of the initial value should occur within 45 minutes. Otherwise the observation was discarded. The results tabulated are the maximal changes observed following each injection. The volume of saline injected was 5 μl., a quantity which had been found to be without effect in a series of twenty preliminary observations. The tensions of cyclopropane and halothane with which the saline was equilibrated were 0.80 and 0.05 atmospheres, respectively. The remainder of the gaseous phase consisted of oxygen and water vapor. The anesthetic tensions employed were equinarcotic in dogs, both representing 3.7 times the alveolar concentrations which were (in a series of 36 animals) just sufficient to permit skin dissection with a hot cautery without causing evidence of pain.

In two cases 5 μl. of a contrast medium was injected through the needle, the circulation arrested and the brain frozen as rapidly as possible (within 3 minutes) with acetone and dry ice, the needle withdrawn and the medulla excised and sectioned with a microtome. In one instance the injectate was India ink; in the other it consisted of Evans' blue dye. The fixed sections were later examined microscopically.

Blood pressure was measured from the femoral artery with a Statham strain gauge. Mean arterial pressures were obtained continuously by electrical damping. The heart rate, arterial blood pressure, mean arterial pressure and stimulation artifacts were recorded by a Grass polygraph. Atropine (0.1 mg./kg.) and dimethyl *d*-tubocurarine (1 mg.) were given intravenously 5–10 minutes before exploration of the brain began.

Results

Responsive Areas Within the Medulla. Little response of the arterial pressure to electrical stimulation was elicited until the electrode was 5 mm. or less from the surface. At this level responses, either pressor or depressor, appeared and increased in magnitude as the needle continued to be withdrawn until a maximum was reached. The peaks were so clearly defined that a further withdrawal of the needle as little as 0.1 mm. frequently diminished the response. In the area 1–2 mm. lateral to and 1–2 mm. rostral to the obex the most frequent response at the deeper levels was pressor. Dorsal to this depressor responses not infrequently appeared. In some animals the first response was depressor, the next pressor, and the last depressor. Depressor areas were most numerous at or near the midline and caudal to the obex. The arrangement of responsive areas tended to be typical of a given animal, appearing repeatedly during the performance of successive punctures. But a well-defined and predictable aggregation of "pressor" and "depressor" representations as described by Alexander [3] was not observed.

Temporal Course of Effects. Following the injection of saline containing either cyclopropane or halothane, maximal effects were noted within five minutes in all cases but one. Peak actions were observed at three minutes in 31, and at one minute in 36 instances. Recovery began as early as five and as late as twenty minutes. In three observations appreciable recovery did not occur, while in four others an inacceptably small degree of reversal was observed (see Methods). There was no apparent difference in the onset of action or in the incidence of recovery when comparing the two agents.

Actions of Cyclopropane. These are summarized in table 1; an illustrative record is graphed in figure 1. The depressant effect of cyclopropane on vasodepressor representations within the medulla oblongata averaged 2.5 times greater than that exerted on pressor representations. There was no significant difference between these areas with respect to the time of maximal depression, the rate of recovery, or the degree of recovery following an injection.

Actions of Halothane are also documented in table 1. It can be observed that the actions of halothane on both pressor and depressor representations were more than twice those observed when cyclopropane was injected into areas from which pressor responses could be elicited.

Actions of Procaine. In two paired experiments one per cent procaine was found to have no effect upon either pressor or depressor responses to medullary stimulation. Two per cent procaine depressed twelve "pressor" areas by 84 per cent on the average, and reduced responses from nine depressor representations by an equal amount (89 per cent) (table 1). Substantial recovery occurred in only one-third of cases during a one-half hour observation period. There was no difference in the incidence of recovery when comparing pressor and depressor representations.

Distribution of the Injectate. Following the injection of 5 μl. of India ink, the pigmented mass occupied a total volume less than 1 μl. and extended down the puncture track a distance of 1 mm. There was no lateral spread of the carbon particles.

TABLE 1. Effects of Cyclopropane, Halothane, and Procaine on the Response of Arterial Pressure to Electrical Stimulation of the Medulla

Agents	No. Observ.	Percentage Change from Control Response:	
		Pressor	Depressor
Cyclopropane Mean (±S.E.)	34	−19.2* (±5.0)	−47.8 (±6.2)
Halothane Mean (±S.E.)	30	−47.1 (±6.1)	−44.3 (±8.5)
Procaine (2%) Mean (±S.E.)	21	−83.5 (±7.3)	−89.1 (±5.6)

* Indicates statistically significant difference between this and all other responses.

Three minutes after the injection of Evans' blue dye, the injectate occupied a volume of approximately 80 μl. symmetrically distributed about the puncture track. The lateral spread was greatest (3 mm.) opposite the point of injection and diminished rapidly with distance, reaching 5–10 per cent of peak density at a distance of 3 mm. There was no apparent disturbance of the normal anatomical

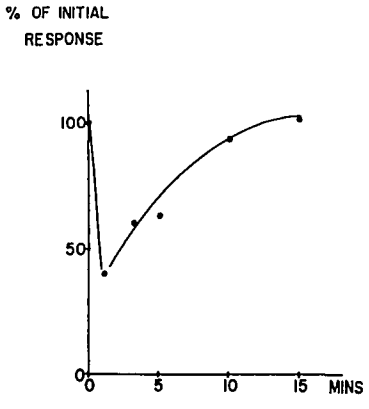

FIG. 1. Effect of microinjection of cyclopropane (in saline) on the response of a vasodepressor representation in a dog's medulla. *Ordinate.* Per cent of initial response of systemic arterial pressure to electrical stimulation of the medulla. *Abscissa.* Time in minutes following the microinjection.

structure within the dyed area surrounding the puncture track.

Discussion

Limitations of the Present Method. The method of local injection of the medulla following decerebration and bilateral vagotomy offers important advantages in the analysis of actions affecting the circulation. The distribution of the agents studied is entirely limited to the area whose responses are to be measured, the effect comes on almost at once, and uninterpretable interactions involving ascending pathways, barostatic reflexes, ganglia, and end-organs are either minimized or avoided.

On the other hand, certain desirable features of whole-body equilibration are lost when agents are given by local injection. The tension of the drug studied can be neither measured nor controlled after the injection has been made, and, since circulatory responses cannot easily be elicited and measured more frequently than once every two minutes, rapid changes in responsiveness resulting from a changing concentration may not be detected. These considerations add to the complexity of interpreting the present study in relation to those of investigators who equilibrate the whole body or whole brain with a single known gas tension, but they are not so difficult to manage as may at first appear.

To begin with the second problem, our results do not establish a significant difference between the responses observed during the 2 minute interval between 3 and 5 minutes following an injection. This suggests that the effective concentration at this time was not changing rapidly.

Evidence from the frozen sections shows that saline flowed outward between the medullary neurones and their processes without disturbing their spatial interrelationships (except as disturbed by the puncture itself), and the final volume of distribution (about twenty times that injected) implies that the pathway followed by the injectate in achieving this distribution was roughly 5 per cent of the total tissue volume, a figure which approximates that estimated for the normal volume of the extracellular fluid within the brain.[4]

Radial flow results in a rapid diminution of concentration with distance (as was found in the experiments with Evans' blue dye), and the implied third power relation between tension and distance suggests that tensions approximating those of the injectate would be found only within a 0.05 mm. distance from the point of injection, and that a tension greater than half that in the injectate would not occur beyond 0.25 mm. In view of the 0.3 mm. tip separation of the electrode and the 0.2 mm. diameter of the central element, it is doubtful that the stimulus could consistently have been applied closer than within 0.2 mm. of the point of maximum drug concentration in these experiments. Thus, the chances of studying the effects of various drug concentrations in the present series were excellent, but the mean effective concentration must have been considerably lower than that injected and the collection of a large number of observations must have obscured any differences in the response resulting from random variations in the spatial relations between the electrode tip and the excitable cells.

Comparing the relative potencies of the agents studied presents certain difficulties. Since the physical properties of saline are not likely to be modified by the solution therein of small amounts of an anesthetic, it is doubtful that the initial distribution of the injectate was systematically different in the experiments with cyclopropane, halothane, or procaine. However, it is probable that the partition ratios of the various substances between brain and saline were different. For example, the ratio for halothane is undoubtedly greater than that for cyclopropane.[5] But this fact can only increase the significance of the observed difference between the responses to these two agents, since the tension of the substance with the higher ratio must diminish more rapidly with increased distance from the point of injection. On the other hand, the spatial distribution of the elements responsible for pressor and depressor responses could have differed in such a way that either area could have been intrinsically more susceptible to the action of any drug, irrespective of the agents' relative actions on the excitable cells themselves. For example, a relatively discrete area, like most of the depressor areas encountered, might be relatively easy to inhibit because of its limited distribution. That this

is not so is suggested, but not proved, by the fact that two agents (halothane and procaine), which were selected essentially at random to represent indifferently depressant agents, were found to depress pressor and depressor representations to an equal extent.

Interpretation of Results. These results are interpreted to mean that all of the anesthetics studied reduce the excitability of certain medullary representations subserving cardiovascular control. Whether neurones, cell processes, synapses, or other elements were most affected is unknown. However, this problem is irrelevant in the present context, since reversible depression could be shown for each anesthetic tested, and at effective concentrations which were probably well within the clinically useful range. In the case of cyclopropane the depression of pressor representations, although highly significant statistically, was significantly less than that caused by the other agents tested. We interpret this to mean that cyclopropane in some way spares medullary pressor representations from the more uniformly depressant effects which typify the responses to procaine and halothane. This conclusion is compatible with our previous hypothesis that "cyclopropane selectively depresses medullary depressor neurones," [2] because the hemodynamic result of sparing pressor and selectively depressing depressor representations should be qualitatively the same. However, it now appears (from comparing the responses to halothane and procaine) that the unique aspect of cyclopropane's actions in the medulla is not a disproportionally large effect on the depressor, but a relatively small effect upon the pressor elements.

The importance of these results depends to a large extent upon whether or not they are consistent with the systemic circulatory actions caused by the same agents when these are confined to the central nervous system. In the dog, the administration of cyclopropane to the head alone has been found to cause systemic arterial hypertension,[2] while halothane [6] and procaine (H. L. Price: unpublished observations) caused systemic hypotension. Since a selective depression of the depressor neurones (as, for example, during carotid occlusion) will provoke systemic arterial hypertension, the remaining question is what happens when the pressor and depressor representations are equally affected. The best available information, from experiments involving brain stem sections, is that as the brain stem is sliced transversely from above downward (*i.e.*, cranio-caudally), the level of systemic blood pressure remains relatively unaffected until the lower pons and upper medulla are entered, when, with succeeding sections, the blood pressure declines progressively until the obex is reached.[3] At this level, only depressor neurones are thought to be active,[3] and the systemic arterial pressure reaches its nadir. A succeeding cut at the level of C_1, which removes the depressor influence, only slightly elevates the blood pressure, and the total reduction consequent upon medullary ablation is therefore large, of the order of 60 mm. of mercury. By analogy an equal depression of pressor and depressor representations would be expected to cause arterial hypotension, and the present results therefore remain consistent with our working hypothesis.

Finally, the results of Markee and Wang [7] and Bartlestone [8] deserve mention. These authors have reported that cyclopropane acts in a way exactly opposite to that which we have proposed. Unfortunately their experiences have so far been published only in preliminary form, and specific comment upon them is therefore impossible. At the moment there appear to be differences in methods, differences in results, and differences in the interpretation of similar results among the various groups of workers in this field.

Summary and Conclusions

This paper reports the results of 85 microinjections of three anesthetic agents upon the response of the vasomotor "center" to electrical stimulation. The results obtained support the view that cyclopropane has a relatively large depressant effect upon the sympathetic depressor representations in the medulla, while the other anesthetics tested (halothane and procaine) act equally upon pressor and depressor representations. It is believed that this unusual action of cyclopropane can explain its ability to cause arterial hypertension in the dog. A note is appended stating that the vasomotor "center" is not a center in the classical sense, but a relatively dispersed ar-

rangement in which responsive sites are numerous, but widely scattered. In addition, the pressor and depressor "areas" described classically do not exist; every animal examined was to an extent unique, and the spatial distribution of pressor and depressor "points" in the various animals examined was not predictable in advance.

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HYPOTENSION Using dogs, blood pressure was reduced either by bleeding or by administration of trimethaphan. In the hemorrhage cases, cardiac output fell, peripheral resistance rose, and acidosis developed. In the trimethaphan cases, output fell, resistance fell, but there was no acidosis. When the shed blood was returned to the animals, the trimethaphan group had a moderate increase in cardiac output, but the group which did not receive a ganglion blocking drug had a greater increase of cardiac output. *(Hopkins, R. W., and Simeone, F. A: Trimethaphan Camsylate in Hemorrhagic Shock, Arch. Surg. 89: 365 (Aug.) 1964.)*

HYPERBARIC BYPASS Fourteen bypasses were carried out on dogs at 3 atmospheres of absolute pressure, and 7 were done at one atmosphere for controls. Maximal, normal, and low flows were studied at normal body temperatures using a small disc oxygenator primed with dextran. Even at low flows the arterial oxygen saturation at 3 atmospheres was 100 per cent and the venous oxygen saturation remained high in contrast to the control values at one atmosphere. The increased oxygenation is reflected in significantly lower lactic acid production at 3 atmospheres. Electrocardiograms and electroencephalograms showed a remarkably good condition while at one atmosphere under similar conditions the electroencephaogram became flat in a few minutes. The P_{CO_2} at 3 atmospheres was slightly higher than in controls, but there was no continuous rise despite the high degree of saturation of hemoglobin with oxygen. Apparently enough CO_2 was removed in solution to prevent significant accumulation. *p*H values at 3 atmospheres were a little lower than in controls. Administration of oxygen at 3 atmospheres provides an enormous safety factor in the technique of priming without blood. *(Meijne, N. G., and others: Extracorporeal Circulation Under High Atmospheric Pressure, Surgery 56: 519 (Sept.) 1964.)*