Reflex Apnea, Bradycardia, and Hypotension Produced by Serotonin and Phenylbiguanide Acting on the Nodose Ganglia of the Cat

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ABSTRACT

Five to 20 µg of phenylbiguanide or serotonin (5-HT) injected into the common carotid arteries of cats (anesthetized with chloralose and urethan) elicited an immediate apnea, usually accompanied by bradycardia and hypotension. The response persisted after ligation of the external carotid and lingual arteries. Since the internal carotid is occluded in the cat, the receptors must be in the distribution of the occipital or ascending pharyngeal arteries. Denervation of the carotid sinus and carotid body, removal of the superior cervical ganglion, section of the vagosympathetic trunk below the nodose ganglion, and extracranial section of cranial nerves IX, XI, and XII did not diminish the response. Section or procaine block of the extracranial supranodose vagus or removal of the nodose ganglion usually abolished the apnea and bradycardia; hypotension still occurred in many cats. Examination of the nodose ganglion by fluorescence microscopy revealed nests of intensely fluorescent cells similar to type 1 carotid body cells. Although previous investigators have reported that veratrum alkaloids or acetylstrophanthidin causes vomiting or bradycardia by an action on the nodose ganglion, this is the first demonstration that a naturally occurring substance (5-HT) stimulates receptors in the nodose ganglion, causing reflex apnea and hypotension in addition to bradycardia.

KEY WORDS 5-hydroxytryptamine vagal body carotid body catecholamine-containing cells sensory receptors thoracic chemoreflex vagus nerve

In the course of determining the effect of intracarotid injections of various chemical substances in the cat, we found that serotonin (5-hydroxytryptamine; 5-HT) and phenylbiguanide (PG) usually elicited immediate apnea, bradycardia, and hypotension. Since this response still occurred after we cut the carotid sinus nerve, it must have been due to an action on receptors other than those in the carotid sinus or carotid body; since it occurred after we also ligated the external carotid artery, the receptors must have received their arterial blood from the occipital or ascending pharyngeal arteries (the internal carotid is usually naturally occluded in the cat). Among the extracranial structures supplied by these two arteries are the larynx, pharynx, muscles of the neck and head, the superior cervical sympathetic ganglia, the nodose ganglia and its closely associated vagal bodies, and extracranial portions of cranial nerves IX–XII (1–2).

We considered the nodose ganglia to be likely sites of receptors sensitive to 5-HT and PG because of the pioneer studies of Borison and associates and the later work of Chai and associates. Borison and Fairbanks (3) were the first to show that chemical substances can excite receptors in the nodose ganglion; they demonstrated that Veriloid, a derivative of veratrum viride, produced emesis by an action on these receptors in the cat. Borison and associates (4) showed that the hypotension
produced by Veriloid was also due, at least in part, to an action on the nodose ganglion. Borison and Sampson (5) later suggested that the receptor site for the emetic action of Veriloid might be the vagal body, a paraganglion closely related to the nodose ganglion. More recently, Chai and associates have reported that acetylstrophanthidin (6) and protoveratrine A (7) cause reflex bradycardia by acting on receptors in the nodose ganglion. The results of our experiments show that 5-HT and PG also excite sensory receptors or afferent fibers in or near the nodose ganglion of the cat and raise important questions about its structure and function.

**Methods**

We studied 24 cats weighing 1.8 to 4.6 kg (mean weight 3.0 kg). The cats were anesthetized initially by 50 mg of chloralose and 250 mg of urethan per kg, given intraperitoneally (0.5 ml/kg of a 0.9% NaCl solution containing 10% chloralose and 50% urethan); when necessary this was supplemented by 1.5 to 3 mg pentobarbital sodium/kg iv. The cats breathed spontaneously through a tracheal cannula and a no. 0 Fleisch pneumotachograph. We recorded on a Grass polygraph the femoral arterial blood pressure (from a catheter connected to a P23G Statham strain gauge), tidal volume (by electrical integration of the signal for air flow), and the percent of CO2 in inspired and expired gas (measured by a Beckman LB-1 gas analyzer).

To expose the structures in the distribution of the carotid arteries, we tied and cut the trachea and esophagus above the tracheal cannula and reflected them, together with the pharynx and larynx, toward the head. For intraarterial injections of chemical substances close to the carotid body, we inserted a polyethylene catheter (PE 50) into the lingual artery and pushed it toward the heart until the tip lay in the common carotid artery 3 to 5 mm before its bifurcation. We ligated the superior thyroid artery, the superior laryngeal artery, the lingual artery where the catheter entered it and the external carotid artery just beyond the origin of the lingual artery. The occipital and ascending pharyngeal arteries were not tied; the internal carotid artery beyond the carotid sinus was not patent in our cats. We loaded the intracarotid catheter (internal volume = 0.25 ml) with the chemical to be tested (0.1 ml) and at the appropriate time flushed it into the common carotid (within 1 to 2 seconds) by injecting 0.6 ml of heparinized saline (1000 U heparin/100 ml 0.9% NaCl). The chemicals used were sodium chloride (reagent grade), sodium cyanide, nicotine bitartrate, phenyl diguanide hydrochloride, serotonin creatinine sulfate, and sodium heparin. Doses were calculated on the basis of the weight of the salt. All were dissolved without a preservative in 0.9% saline.

In every experiment, sodium cyanide and nicotine were each injected intraarterially in a dose that stimulated the carotid body; when repeated after the ipsilateral carotid nerve was cut, the same dose of cyanide produced no effect and nicotine elicited either no effect or a brief apnea (6 of 14 cats) and bradycardia (3 of 14 cats).

At the end of some experiments, we diluted silicone rubber compound (G.E. RTV-60) with CF 1025 silicone fluid (8) and injected it into the common carotid artery; we then removed the tissues of the neck, cleared them using the Spalteholz method (9) and examined the specimens under a dissecting microscope to determine the arterial blood supply of the nodose and sympathetic ganglia.

In some experiments a polyethylene tube was placed in the external carotid artery and connected to a Statham P23G strain gauge so that we could record femoral and external carotid blood pressures simultaneously during occlusion of the ipsilateral and contralateral common carotid arteries. These tracings provided us with information on the existence of connections between the vertebral artery and the occipital-ascending pharyngeal arteries in the cat.

**Results**

A typical response to the intracarotid injection of 5-HT and of PG before and after section of the ipsilateral carotid sinus nerve is shown in Figure 1. Before section, injection of each chemical produced immediate apnea, bradycardia and hypotension; after section, the response was unchanged or increased. Because carotid denervation did not diminish the response, we are reporting only the effects produced by intracarotid injections after the carotid sinus nerve was cut. No tachyphylaxis followed repeated doses in the ranges used (5-20 µg) when at least 2 minutes elapsed between injections.

Intracarotid 5-HT or PG produced apnea or a marked decrease in tidal volume in 23 of 24 cats. In 16 cats in which one or both vagosympathetic trunks were intact, the injections produced bradycardia and hypotension as well. Because of the possibility that

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Effects of intracarotid injections before and after cutting the carotid sinus nerve. Top: Carotid sinus nerve intact on side of injection. Bottom: Same cat. Carotid sinus nerve cut.

TV = respiratory tidal volume; BP = femoral arterial blood pressure; PG = 5 μg of phenylephrine; 5-HT = 5 μg of 5-hydroxytryptamine. Injections of PG and 5-HT were made (at black bars) into the right common carotid artery just before its bifurcation; the external carotid artery on that side was tied several millimeters above the origin of the occipital and ascending pharyngeal arteries.

Bradycardia might result from direct activation of efferent cardio-inhibitory vagal fibers passing through or around the nodose ganglion, we injected 5-HT and PG into the carotid artery after cutting the ipsilateral vagosympathetic trunk just below the nodose ganglion, leaving the contralateral vagus trunk intact. The injections still produced bradycardia in six of the eight cats tested; this bradycardia must have been due to excitation of sensory receptors, afferent fibers, central neurons, or all three. When both vagosympathetic trunks were cut on the cardiac side of the nodose ganglia, no bradycardia occurred after the injection of 5-HT or PG, but apnea and hypotension still occurred in each of nine cats (Fig. 2). That this hypotension was due to peripheral dilation in the systemic circulation was shown by perfusing a hind limb with a constant flow of blood using a Sarns pump; intracarotid injections of 5-HT or PG led to a decrease in perfusion pressure in the limb. This vasodilation was not prevented by previous injection of 1 mg atropine/kg iv.

We then determined the location of the structures sensitive to 5-HT and PG. Section of cranial nerves IX, XI, and XII and the postganglionic sympathetic trunk just before they enter the cranium (Fig. 3) did not alter the response to intracarotid injections of 5-HT or PG. However, sectioning the ipsilateral supranodose vagus (Fig. 2) or blocking it with 2% procaine (Fig. 4) abolished the respiratory response to a previously effective dose of 5-HT or PG. In one of the remaining three cats, the typical response was completely abolished by procaine block of the ipsilateral supranodose vagus and returned when the block wore off, but was not abolished later by excision of the nodose ganglion; however, between the time of the block and the excision, the external carotid
Effects of intracarotid injections before and after cutting the supranodose vagi. Top: Both vagosympathetic trunks had been cut on the cardiac side of the nodose ganglia and both carotid sinus nerves had been severed. Bottom: Same cat after section of both supranodose vagi. Abbreviations and procedures same as in legend for Figure 1 except that 10 μg of PG and of 5-HT were given.

Arteries and nerves in the region of the carotid bifurcation of the cat. The diagram shows the site of section of the cervical vagosympathetic trunk, of the postganglionic sympathetic trunk, of cranial nerves IX, XI, and XII and of branches of the superior cervical sympathetic and nodose ganglia. The apnea caused by intracarotid injection of PG and 5-HT persisted after section of each of these nerves but not after cutting the supranodose vagus. Sympath. Gang. = superior cervical sympathetic ganglion; Nodose Gang. = nodose ganglion; S.L. = superior laryngeal nerve; was ligated, so that now the same dose of 5-HT or PG would result in a higher concentration in the carotid blood. In the second cat, the response was not abolished by section of the supranodose vagus but was abolished by excision of the nodose ganglion. In the third cat, the response still occurred after section of both supranodose vagi.

In four cats in which both cervical vagosympathetic trunks were intact, intracarotid injections of 5-HT and of PG produced bradycardia before but not after block or section of the ipsilateral supranodose vagus. In 11 of 13 cats, 5-HT and PG produced an immediate hypotension before the supranodose vagus was cut and still produced a
Similar hypotension after its section in 6.

These experiments indicate that in most cats, the typical response to small, intracarotid doses of 5-HT and PG occurs only when the nodose ganglion and the supranodose vagus are intact. This does not prove that the nodose ganglion and supranodose vagus are the site of extracranial receptors, for these structures might serve only as a path to the brain for afferent fibers coming from extracranial receptors elsewhere. It is known that fibers from the superior cervical ganglion (10) and from the hypoglossal nerve (11) enter the nodose ganglion of the cat and that the common carotid (12), the superior laryngeal, and the pharyngeal nerves join the vagus at or just above the level of the nodose ganglion. Therefore, we excised the superior cervical sympathetic ganglion, sectioned the fine fibers connecting cranial nerve XII and the nodose ganglion and sectioned the superior laryngeal, pharyngeal and common carotid nerves; none of these procedures diminished the response to 5-HT or PG. We conclude from these studies that the extracranial receptors are in or near the nodose ganglion. This conclusion was strengthened by two experiments in which we were able to occlude or ligate fine arterial branches going only to the nodose ganglion. In each case, previously effective doses of 5-HT now produced no effect.

Dr. Donald McDonald prepared a nodose ganglion of one cat for fluorescence microscopy using a technique (13) that converts catecholamines to fluorescent compounds. The ganglion contained three circumscribed groups of small, intensely fluorescent cells among the cell bodies of the sensory neurons (Fig. 5). The fluorescent cells are morphologically similar to the type 1 cells of the carotid body, but we do not know if these are the cells that are sensitive to 5-HT and PG.

The results in some of our cats suggested that 5-HT and PG act on intracranial receptors as well; after block or section of both supranodose vagi, these chemicals still caused apnea in 2 of 13 cats and hypotension in 6 of 11 cats. Our preparations injected with
silicone rubber (Fig. 6) showed that branches of the occipital and pharyngeal arteries, after supplying the nodose ganglion, enter the suprano-dose vagal trunk and probably supply the jugular ganglion of the vagus and some other intracranial structures; however, in each of four cats, injection of 2% procaine into the suprano-dose vagus abolished the responses to intracarotid 5-HT and PG although the vascular supply to the trunk was intact.

After we tied the external carotid artery (thus eliminating communication with the...
Photograph of arterial supply of nodose ganglion. Silicone rubber was injected into the common carotid artery (C.C.), and it and surrounding tissues were cleared using the Spalteholtz technique. The material used does not fill capillaries or pass into venules. Branches of the occipital and ascending pharyngeal arteries supply the carotid body (C.B.), superior cervical sympathetic ganglion (Sym.), nodose ganglion (Nodose) and other nerve trunks in this region.

Internal maxillary artery, believed by some (14) to be the only important arterial connection between the common carotid and the circle of Willis), occlusion of the ipsilateral common carotid artery reduced blood pressure above the clamp to only 90, 70, and 70 mm Hg in three of four cats; in only one did the blood pressure in the "isolated" segment fall to 20 mm Hg. Since a high pressure could be maintained in the isolated segment even with both common carotids clamped, either the occipital (15) or ascending pharyngeal artery (1, 15) must have communicated with either the vertebral artery or the circle of Willis. We have not tried to determine where the intracranial receptors are in the distribution of the occipital-ascending pharyngeal arteries.

Because 5-HT and PG, acting on structures in or on the nodose ganglion, caused reflex apnea and hypotension, we tried to determine whether these structures exert a tonic effect on breathing and blood pressure. In three cats, we cut the superior laryngeal and pharyngeal nerves and each vagosympathetic trunk just below the nodose ganglion; when the cat's breathing was again stable (though slower and deeper), we cut both vagal trunks just above the nodose ganglia. In one cat, apnea (32 seconds) and hypertension (20 mm Hg) occurred; in a second, apnea (12 seconds) occurred with no hypertension. In a third, apnea occurred for 6 seconds after cutting the second supranodose vagus but blood pressure did not change.

In dogs whose carotid sinus nerves are intact, intracarotid injections of 5-HT and PG produce hyperpnea by stimulating the carotid bodies (16). We injected 5-HT and PG into the common carotid arteries of two dogs whose carotid nerves were cut, to determine whether these substances also act on receptors
in or near the nodose ganglia. Both agents produced a transient increase in tidal volume and an increase followed by a decrease in blood pressure. The response was not changed by supranodose vagotomy. In neither dog did the response resemble the combination of apnea-bradycardia-hypotension typically seen in the cat.

**Discussion**

These experiments prove that two chemical substances, 5-HT and PG, injected into the arterial blood supply of the nodose ganglia of the cat, activate receptors within or on the nodose ganglia and so cause reflex apnea, bradycardia, hypotension, and vasodilation in the limbs. Previous investigators have reported that some chemicals have an action on or near the nodose ganglia. Borison and associates (3, 4) found that the emetic action of Verlolid and part of its hypotensive action were due to an effect on receptors in or near the nodose ganglia of the cat. Tanaka and Kanno reported similar findings using protoveratrine in cats and rabbits (17). Chai and Wang (7) showed that intracarotid injections of protoveratrine A caused reflex bradycardia and hypotension in the cat and concluded that the receptors were in the nodose ganglia or neighboring structures. Chai and associates (6) showed that intracarotid injections of acetylstrophanthidin also caused bradycardia and hypotension; they attributed the immediate response to an action on the carotid sinus and the late response (delayed 1 to 5 minutes) to an action on the nodose ganglia. Borison and Sampson (5) raised the question whether the chemosensitive structure might be the vagal body (paraganglion intravagale) which lies in, on, or near each nodose ganglion in some birds and in the rabbit (18, 19) and man (20). Our experiments are the first to show that a naturally occurring and physiologically important substance, serotonin, can activate receptors in or on the nodose ganglion; they are also the first to demonstrate that apnea is an integral part of this reflex.

The triad of reflex apnea, bradycardia, and hypotension has been previously described as resulting from stimulation of receptors (a) in the pulmonary and coronary circulations by chemical substances (21), including serotonin (22), (b) in the carotid sinus and aortic arch by increased pressure, (c) in the nose by immersion, and (d) in the larynx by mechanical or chemical stimulation. We have now described it from stimulation of receptors in the nodose ganglion. On this account it was important to be sure that the substances we injected into the common carotid artery through a catheter inserted through the lingual artery were not acting on receptors for the pulmonary or coronary chemoreflex. (An action on receptors in the carotid sinus and larynx was ruled out by appropriate nerve sections.) If the chemicals acted on the receptors for the pulmonary chemoreflex after recirculation, the response would have been delayed 8 to 16 seconds; however, it began within 1 to 3 seconds. If the chemicals activated receptors for the coronary chemoreflex as a result of retrograde injection down the common carotid, no response should have occurred after bilateral midcervical vagotomy, since the afferent fibers for the coronary chemoreflex run in the vagi; although bilateral vagotomy abolished the bradycardia (because the efferent parasympathetic fibers to the heart were cut), it did not diminish the reflex apnea or hypotension produced by intracarotid 5-HT and PG.

Previous workers (23-25) who have injected 5-HT into the right ventricle of cats have noted that striking apnea, bradycardia, and hypotension occurred when the vagi were intact but not, as a rule, after they were blocked or severed. This argued against the existence of another group of serotonin-sensitive receptors in the distribution of the occipital and ascending pharyngeal arteries. However, earlier workers were unaware that the lungs and pulmonary circulation have a biochemical function (26) and that 90%-95% of serotonin in the pulmonary arterial blood is taken up during a single passage through the lungs (27). Thus, after injection of serotonin into the right heart, its concentration in carotid arterial blood is far lower than that in pulmonary arterial blood.
Earlier workers disagreed on whether 5-HT and PG, injected into the left ventricle or ascending aorta (and so not subject to biochemical transformation in the pulmonary circulation) still produced apnea and hypotension when the vagi were cut. Several (22-25) did note that apnea still occurred in some cats, but these investigators were either unable to locate the receptors or attributed the apnea to a central action. We have now found that the response to intracarotid injection is due in most cats to an action on receptors in or near the nodose ganglion but, as in the earlier experience, apnea and hypotension still occur in an occasional cat after elimination of known extracranial receptors. It is likely that intracranial structures also respond to suitable concentrations of 5-HT and PG.

There has been no thorough study of the structure of the jugular and nodose ganglia of the vagus nerve in the cat since that of Ranson and associates (28). They were convinced that the nodose ganglion was a sensory ganglion without an appreciable admixture of sympathetic ganglion cells, and they made no mention of special chemoreceptor-like cells. Muratori in 1932 (19) noted the presence of chemoreceptor-like cells in the nodose ganglia of birds, and White in 1935 (29) found similar cells just below the nodose ganglia of man within the perineurium of the vagus nerve. Birrell (30) named this the vagal body and noted that the cells were distributed in multiple small foci at the level of the nodose ganglion. Seto and associates (20) found small groups of cells, similar to carotid body cells but about half their size, scattered throughout the nodose ganglia of man, and Lattes (31) also described cells resembling those of the carotid body in the human nodose ganglia. The nests of catecholamine-containing cells that we found in the nodose ganglion may be the same cells described by previous workers who used conventional staining techniques. Muryobayashi and associates (32) found abundant fluorescent fibers in the cervical vagal trunk of the cat but very few in the nodose ganglion; they did not mention nests of fluorescent cells in the ganglion.

Since serotonin and phenylbiguanide produce little or no effective stimulation of carotid chemoreceptors in the cat, it is unlikely that the fluorescent cells in the nodose ganglion are displaced carotid body cells. And these fluorescent cells may have nothing to do with chemoreception; 5-HT and PG may stimulate sensory ganglion cells themselves, the rootlets of the central or peripheral fibers emerging from the unipolar sensory ganglion cells or other chemoreceptor cells or nerve endings. We believe, however, that the chemosensitive structures that initiate this reflex must be mainly in or on the nodose ganglia and not distributed widely along many fibers of the vagus, (as described for the intrathoracic vagus in the dog by Coleridge et al. (33) and for the abdominal vagus in the hamster by Chen and Yates (34); this is because intracarotid injections of 5-HT produced the same response before and after the infranodose vagus was cut and produced no response when the arterial blood supply to the supranodose vagus was intact but the nerve fibers were blocked by procaine.

The discovery of chemosensitive areas in the nodose ganglia adds to existing knowledge that sensory fibers enter the vagi at and above the nodose ganglia and emphasizes the need for physiologists to specify the level at which the vagi are cut, blocked, or stimulated in their experimental work; it is now clear that midcervical vagotomy does not block all vagal sensory input to the brain but only that originating caudal to the nodose ganglia.

We do not know the physiological role of serotonin-sensitive receptors in the nodose ganglion. Borison et al. (4) excised both nodose ganglia in a few cats whose cervical vagi had been cut previously; they noted that this procedure "occasionally resulted in a small rise in blood pressure of 10-15 mm Hg"; they did not measure respiration. In three cats in which we performed supranodose vagotomy after infranodose vagotomy, blood pressure rose 20 mm Hg in one but did not change in the other two; brief apnea (6, 12 and 32 seconds) occurred in all three cats. (The apnea might have been due to injury...
potentials produced by cutting fibers from pulmonary stretch receptors, though a similar response did not occur when both infranodose vagi were severed.) In our procedure we had cut the superior laryngeal and pharyngeal nerves before cutting the supranodose vagi; since Borison and associates had not, they may have seen the effect of eliminating afferent input from the larynx and pharynx to the nodose ganglia rather than the effect of eliminating intrinsic activity of the ganglia. Receptors in or near the nodose ganglia may have no tonic activity but respond only when chemical substances, not normally free in blood, excite them. And they may be physiologically or pharmacologically active only in the cat although they have been described anatomically in birds and rabbits and in man. We did not obtain a reflex response from the nodose ganglion in two dogs when we injected 5-HT or PG into the arterial blood supplying the ganglion; however, the chemoreflex responses to 5-HT and PG differ in several ways in the cat and dog. In the cat, these two chemicals produce little or no activation of the carotid body but marked stimulation of the pulmonary and coronary chemoreflexes and of the nodose ganglia; in the dog, they produce consistent stimulation of the carotid body and little or no activation of the pulmonary and coronary chemoreflexes or of the nodose ganglia.

In the last decade, histologists have discovered nests of chemoreceptorlike cells associated with the vagi and sympathetic nerve trunks; it will be important to learn whether they have a sensory, endocrine, or neurotransmitter function.

References


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