Effects of Protoveratrine, Serotonin and ATP on Afferent and Splanchnic Nerve Activity

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Splanchnic outflow was studied along with carotid and aortic presso- and chemoreceptor inflow in cats under a variety of conditions. Splanchnic activity was increased by a decrease in presso- or increase in chemoreceptor activity and vice-versa. Blood pressure rose following splanchnic activation. Protoveratrine and serotonin, reported to activate the "coronary chemoreflex," depressed completely splanchnic outflow, without enhancing pressoreceptor activity. Prolonged hypotension from protoveratrine coincided with partial return of splanchnic activity and tonic discharge of pressoreceptors. ATP increased splanchnic activity simultaneously with the hypotension and it is suggested that it be excluded from the list of drugs eliciting the "coronary chemoreflex."

Except for an early paper by Gernandt and coworkers, no studies have been reported, in which action potentials from the splanchnic nerves have been recorded under various experimental conditions; no information yet exists on direct relation between carotid and aortic afferent activity and efferent splanchnic activity in hypotension induced by agents activating afferent vascular arcs, such as the veratrum alkaloids, serotonin, and adenosine triphosphate. Those drugs are reported to elicit a "coronary chemoreflex" (v. Bezold-Jarisch effect) but considerable controversy exists with regard to the participation of the carotid and aortic afferents and the efferent pathways involved. Such studies form the basis of this report.

Methods

The experiments were done on 60 cats lightly anesthetized with chloralose (90 mg./Kg.) and urethane (300 mg./Kg.). The animals were artificially ventilated with air, just enough to suppress spontaneous respiratory movements, and then neuromuscular blockade was accomplished with decamethonium, or with tubocurarine in some cases. Skeletal muscle paralysis was necessary to allow differentiation of movement artefacts from true splanchnic potentials and for the "asphyxia" test, to be described below.

Preparation of the right carotid sinus nerve was done according to classic methods, and only occasionally were fibers selectively eliminated. The left depressor nerve was identified at the base of the neck, before it joins the vagus, and usually dissected free from the vagus for some distance centrally. The right major splanchnic nerve was identified (through an extraperitoneal approach, as described by Gernandt and coworkers) behind the kidney as it emerges from the crus of the diaphragm, and in the majority of cases followed for one or 2 cm. into the thoracic cage. The last rib was partially resected during this procedure, and this almost always resulted in right pneumothorax. The nerve was cut just before its entry into the celiac ganglion and recording was done therefore from preganglionic fibers. The sheaths were removed from all three nerves (carotid, depressor, and major splanchnic) to improve the signal-to-noise ratio.

In view of the properties of the preganglionic sympathetic fibers, no attempt was made to prepare and record from single fibers. The nerves were kept in oil, and amplification and recording were done as described elsewhere. Recording from the splanchnics offered technical difficulties because of the admixture of electrocardiogram usually present and possibly also from unknown intraabdominal pressure changes. By suitable rotation of the electrodes it was possible to eliminate ECG interference in about half the cases; the remaining cases offered the possibility of studying effects of drugs on ECG as well as on the splanchnic outflow. Afferent impulses in the splanchnics were met on occasion, originating probably from the injured part of the nerve; their features (A fibers) and their relatively small numbers did not pose special problems in interpreting splanchnic records.

Blood pressure through one femoral was recorded on a smoked drum; the other femoral artery was cannulated in experiments during which the effects of hemorrhage were studied. Stimulation of splanchnic activity by excitant drugs was achieved either by intravenous administration or more frequently by close intrarterial injection.
EFFECT OF DRUGS ON SPLANCHNIC ACTIVITY

Fig. 1. Both strips are obtained from a different animal and contain from above downwards: timer (in $\frac{1}{30}$ second), action potentials of carotid sinus nerve and action potentials of a branch of the major splanchnic nerve. Above: "pulse synchronous" type of splanchnic activity; an ECG pickup can be seen in the tracing of the splanchnic activity (the hump between the first and the second grouped discharges is a QRS complex). Calibration for both curves at upper left corner 50 $\mu$V. Below: "continuous" type of splanchnic activity; the tracing of the carotid sinus nerve bears a recognizable ECG and represents the activity of one large pressoreceptor fiber.

Injections (by way of the lingual artery) into the left carotid area, the nerve of which was connected with the central nervous system. In the first case the effects on splanchnic outflow resulted from action both on chemoreceptors and on the central nervous system, whereas in the latter the effects (initially at least) were due to a reflex action from stimulation of one carotid area. The left intracarotid injection was designated as "contralateral" and it did not usually alter the activity of the right carotid nerve, which was used for recording purposes.

In the vast majority of experiments the activity of the carotid sinus (above) and splanchnic (below) nerves have been recorded by a dual beam oscilloscope. In some instances carotid sinus and aortic arch nerve potentials were obtained, or aortic and splanchnic, wherever specific information with regard to the interrelation of these nerves was necessary. The timer was interrupted during the period of injections, thus permitting accurate determination of latency of responses.

The following excitant substances were injected intrarterially to the "contralateral" (left) carotid area: acetylcholine bromide in total doses of 1.0 to 5 $\mu$g.; sodium cyanide in doses of 25 to 50 $\mu$g.; lobeline sulfate in doses of 10 to 50 $\mu$g.; all three were diluted to a constant volume of 0.5 ml. with saline.

In addition to the above substances, increase of splanchnic outflow was accomplished by slow bleeding of the animal to 70 or 50 mm. Hg into an automatic stabilizing reservoir.

An "asphyxia" test was done on the "curarized" animals by stopping the ventilation for 15 to 60 seconds. The resulting blood pressure rise proved a valuable method for studying the excitability and responsiveness of autonomic centers. Protoveratrine maleate A or B, 5-hydroxytryptamine (serotonin) creatinine sulfate and disodium adenosine triphosphate (ATP) were diluted with saline to a volume of two to three ml. and administered intravenously into the femoral or external jugular vein.

Results

Normal Pattern of Splanchnic Activity:
Figure 1 shows the two most common patterns of splanchnic activity. In about two-thirds of the animals studied the pattern was that of the upper strip. This consists of pulse-synchronous discharges, having maximal intensity at about the end of each carotid pressoreceptor discharge. This relationship is not fixed and there is a shift from cycle to cycle of the maximum density of splanchnic discharge. The conduction velocity of these impulses is considerably slower than that of the pressoreceptor impulses, this being in accordance with the fact that they stem from preganglionic fibers and thus should be classified as B type; C impulses have also been frequently met. The large positive after-potentials, obvious when these impulses appear in synchronized bursts, give to the curve the wavy form.

A second pattern of activity is the "continuous" type, seen in about 30 per cent of the cases and is illustrated in the lower strip; the splanchnic discharge is continuous throughout and has no relationship to the pressoreceptor impulses. As the spikes do not occur in salvoes positive after-potentials are not obvious and the short time constant of the amplifier cancels them out. We have not seen in the cat splanchnic discharges synchronous with any of the respiratory phases, as reported elsewhere.† Experiments on dogs (not discussed here) have shown a considerably higher incidence of "continuous" and "respiratory" types of splanchnic discharge. Mixed types of splanchnic outflow have been observed in about 10% of the cases, composed of pulse-synchronous and continuous types of discharge.

In the following study of altered splanchnic

* Kindly supplied by Dr. K. Kohlstaedt of the Eli Lilly Co. of Indianapolis, Ind.
† Kindly supplied by Dr. R. K. Richards of the Abbott Laboratories, North Chicago, Ill.
outflow we will discuss mainly the changes observed in the more common or "pulse-synchronous" type of splanchnic activity. Some examples of "continuous" splanchnic outflow will be also presented.

**Increase of Splanchnic Outflow by Pressor and Chemoreceptor Mechanisms**

(A) Hemorrhagic Hypotension. During hemorrhage the splanchnic activity increases proportionately with the hypotension. At moderately hypotensive levels, obtained by slow bleeding, slight overventilation is adequate to prevent any pressoreceptor excitation, so that decreased chemoreceptor activity is probably solely responsible for the increased splanchnic discharge; the somewhat different results of Landgren and Neil are probably due to the more rapid bleeding used in their experiments. The changes in the splanchnic pattern are qualitative as well as quantitative. Previously irregular activity may become pulse synchronous or continuous, usually with exacerbations during each pulse (fig. 2B). When a prominent pulse-synchronous activity is elicited after bleeding, the maximal density of discharge usually follows the pressoreceptor burst, or sometimes coincides with it, especially at low pressures. The shift of splanchnic discharges from pulse to pulse is minimal during mild hemorrhage.

Chemoreceptor activity appears at levels below 70 mm. Hg, in the form of continuously discharging small spikes, double contoured at their bases, possessing a slower conduction rate; this is associated with further enhancement of the splanchnic discharge (fig. 2C);

**Figure 2.** A: control record, B. P. 155 mm. Hg; weak splanchnic discharge. B: 2½ minutes after onset of bleeding (20 ml.) into a reservoir, B. P. 115 mm. Hg; marked increase of splanchnic activity, accentuated during each systole. C: 11 min. after onset of bleeding (total 45 ml.). B. P. 50 mm. Hg chemoreceptors active and almost complete disappearance of pressoreceptors; maximal splanchnic discharge, continuous.

**Figure 3.** Above: control record, B. P. 105 mm. Hg. Below: one minute after intravenous administration of 1 mg. acetylcholine bromide. B. P. 70 mm. Hg. Bradycardia; chemoreceptor stimulation not any more obvious. Splanchnic discharge continuous.

**Figure 4.** Intraarterial injection of 25 mg. lobeline sulfate in 0.5 ml. saline to the left carotid artery. Recording from the right carotid and splanchnic nerves. Animal somewhat underventilated (chemoreceptors active). A: at interruption of timer, beginning of injection. Weak, pulse synchronous splanchnic discharge; a QRS pick-up is obvious. B, 0.13 seconds after A: initial depression of activity followed by (arrow) a heavy discharge with latent period of 1.2 seconds. C, 0.61 seconds after B: gradual decrease of the reflex discharge. Note unaltered right carotid activity during the injection period (3.5 seconds), which ends in strip C. B. P. 120 mm. Hg throughout.
pulse related exacerbations of splanchnic discharge disappear when the pressoreceptor activity becomes almost absent.

(B) Peripheral Vasodilators. Figure 3 shows an example of increased splanchnic discharge following the intravenous injection of one \( \mu g \) per Kg. of acetylcholine, at a time when the chemoreceptor excitation by acetylcholine was no longer obvious. The increase is initially more intense due to the chemoreceptor stimulation but the hypotensive effects (and increased splanchnic discharge) last considerably longer than the chemoreceptor excitation.

(C) Chemoreceptor Excitants. Increase in splanchnic outflow was also achieved by increase of the chemoreceptor inflow, without change of the pressoreceptor inflow. Such an increase can best be obtained by close intraarterial injection of chemoreceptor stimulants into the “contralateral” carotid body area (fig. 4). The splanchnic discharges are depressed during the initial phase of the injection, perhaps because of distention of the walls of the functionally innervated sinus. Then, as chemoreceptor stimulation develops, one to two seconds after the beginning of lobeline, acetylcholine or potassium chloride injection and six to eight seconds after sodium cyanide injections, as described elsewhere, a very intense splanchnic discharge occurs, which is usually continuous for several seconds, especially so with hypotensive doses of acetylcholine. The splanchnic activation results into a moderate blood pressure rise, if the injected excitant is not itself a hypotensive agent.

(D) Asphyxia. Interruption of artificial respiration in the paralyzed animals leads to a general chemoreceptor stimulation, resulting in a very intense splanchnic outflow. As reported by Gernandt and associates the increase of splanchnic activity occurs stepwise with alternating periods of normal and greatly increased activity. The increased outflow is achieved by activation of larger numbers and more synchronized discharges of efferent units, initially retaining their pulse relationship, so that the curve differs from the controls only with respect to voltage (fig. 5B). Later on, the active units fire during progressively greater parts of each cardiac cycle, so that, with prolonged periods of asphyxia, the splanchnic discharge becomes continuous (fig. 5C). The resulting gradual increase in blood pressure is indicated in the carotid neurogram by the larger number of active pressoreceptor fibers and the more frequent firing of those active at normotensive levels; also obvious is the enhancement of chemoreceptor activity in such a condition.

**Decrease of Splanchnic Outflow by Presso- and Chemoreceptor Mechanisms**

(A) Post-anoxic Depression. Five to 10 seconds after readministration of artificial ventilation in the asphyxiated animal, the splanchnic discharges decrease very rapidly and complete inactivity is reached, lasting 15 to 20 seconds. This decrease is already obvious at a time when chemoreceptor activity is still enhanced,
and if the conditions in the splanchnic centers approximate those of the carotid body, one should expect the decreased activity to occur much later. Post-anoxic depression, potentiated by a still enhanced pressoreceptor activity and a rapidly waning chemoreceptor support, may eliminate splanchnic outflow.

(B) **Pressor Agents.** A decrease or abolition of splanchnic activity occurs regularly with infusions of pressor agents, even with a rise in blood pressure of 5 mm. Hg. The splanchnic depression continues as long as the blood pressure is kept 20 mm. Hg or more above the control levels. Excitation of pressoreceptor and inhibition of chemoreceptor mechanisms are the major factors in this response. Similarly, rapid intraarterial injections in the "contralateral" carotid sinus of 0.5 to one ml. of saline, or even chemoreceptor excitants, lead to a momentary decrease or abolition of splanchnic discharges before the reflex splanchnic excitation occurs (fig. 4). Increase of intrasinusal pressure by the injection is probably the cause of this reaction, but the splanchnic inhibition obtained by this way seldom lasts more than one to two seconds; a more prolonged inhibition of splanchnic outflow can be seen with the intravenous injection into the jugular vein of volumes of 2 to 3 ml. of saline. The resulting brief rise in blood pressure (5 to 10 mm. Hg for 5 to 10 seconds) leads through general pressoreceptor activation to a considerable decrease in splanchnic outflow and a slight bradycardia (fig. 8B).

**Rhythmic (Vasomotor) Blood Pressure Waves**

Rhythmic fluctuations of blood pressure independent of respiratory phases were observed occasionally in animals with vagi intact, but vagotomized animals displayed these waves more regularly. On occasion these waves were of large amplitude and very low frequency (two to three per minute).

Figure 6 shows in a non-vagotomized cat the carotid sinus and splanchnic activity at the bottom and the peak of a wave of exceptional amplitude; the blood pressure fluctuated between 80 and 150 mm. Hg, and 1 to 2 such cycle per minute occurred for a considerable period of time. In the upper strip, the mean blood pressure was 80 mm. Hg and the heart rate 215 per minute. A very weak carotid pressoreceptor discharge was obvious with each pulse and also a moderate amount of chemoreceptor activity (animal ventilated). The splanchnic discharges were of the continuous type, and several waves consisting of synchronized active units and positive after-potentials were obvious. The lower strip was obtained 30 seconds later, at a blood pressure of 150 mm. Hg. The heart rate was then 200 per min. and the carotid pressoreceptor activity was greatly enhanced; the chemoreceptor activity decreased and actually most of the small, fast background spikes were pressoreceptor in nature. The activity of the splanchnics was negligible, with only two groups of small discharges immediately following the QRS complexes of the first and second beat. The decreased sympathetic activity was responsible for the transient nature of the blood pressure rise; the pressure declined rather rapidly and the cyclic change was repeated.

Two observations in figure 6 might be emphasized: first, the fact that although the animal was under artificial respiration a moderate amount of chemoreceptor activity existed at hypotensive levels, which was abolished when the blood pressure rose to 150 mm. Hg, although the rate of ventilation did not change; and second, that doubling of the blood pressure and decreasing the chemoreceptor inflow slowed the heart rate only from 215 to 200 per minute.
Comparison of Aortic Arch and Splanchnic Nerve Activity

The pressoreceptor inflow from the aortic area seemed larger and the chemoreceptor much smaller than that of the carotid area; the temporal relationship of the splanchnic outflow to the aortic inflow was still less clear-cut than the splanchnic-carotid relationship.

Hypotensive Action of Reflexly Acting Agents

1. Protoveratrines. Protoveratrine A and B are the most widely used pure alkaloids in the treatment of hypertension, and their effects in man have been thoroughly investigated. We studied their action with the following objectives: to determine the extent of the participation of carotid and aortic pressoreceptors in the hypotension induced by their intravenous administration and to determine the behavior of the splanchnic efferent activity during protoveratrine-induced hypotension.

The intravenous injection of 3.0 to 5.0 \( \mu \text{g} \) per Kg. of protoveratrine (A and B) in the cat, intact or with severed carotid nerves resulted within 5 to 10 seconds in an abrupt bradycardia and hypotension (minimal blood pressure level reached within another 10 to 20 seconds), although in about a third of the cases both a slower onset and a gradual hypotension were observed. Latencies longer than one minute and the late developing response of the human\(^8\) were not observed in the non-vagotomized cat. The recovery was slow and the blood pressure (and moderate bradycardia) remained at levels of two thirds of the original values for 30 to 90 minutes.

Five vagotomized cats responded to protoveratrine with a longer latent period (two to four minutes) and usually with a gradual hypotension to a level half that expected in non-vagotomized animals; bradycardia was also missing in these animals. Three cats with vagi and carotid nerves sectioned, responded to protoveratrine with a rise in blood pressure.

During the initial blood pressure fall carotid and aortic pressoreceptor spikes decreased rapidly and splanchnic discharge was reduced in amplitude and frequency. The decreased amplitude of the splanchnic bursts (fewer active units at a time) was the first observable change, along with a minimal spreading of each grouped discharge. Following this, a partial blockade occurred, that is, discharges appearing after each other heart beat, rapidly followed by a complete quiescence, in spite of the blood pressure fall (fig. 7).

Pressoreceptor activity became increased only during the partial recovery from the initial hypotension 2 to 4 minutes after the injection. Initially this appeared as a prolongation of the pressoreceptor burst with each pulse, so that it lasted for most part of the diastole; occasionally 1 or 2 high voltage spikes were already active continuously. A short period of asphyxia precipitated the appearance of continuously firing units. The effects of anoxia and protoveratrine on pressoreceptors seemed to be similar and additive.\(^9\) Increase of chemoreceptor activity was also present, but was overshadowed by the very intense pressoreceptor traffic. Chemoreceptors discharged for 2 to 3 minutes after readministration of ventilation in asphyxial tests in
contrast to the rapid (15 to 20 seconds) return to normal in control runs.

The splanchnic activity gradually reappeared during the period of prolonged hypotension but the pulse dependency was barely obvious, the discharge being almost continuous; another feature was a decreased synchronization and/or reduction in conduction speed. Unitary activity was not easily detectable at this stage, so that the second alternative could not be proved. One can, however, see the difference in the spread of excitation from the proximal to the distal electrode by comparing the top and the bottom records of splanchnic activity in figure 7.

During the early hypotensive period, chemoreceptor excitation (left intracarotid injection of stimulants) failed entirely to arouse splanchnic discharges; a further slowing of the heart rate by such excitation became also obvious within one to two seconds, whereas normally these substances do not exert negative chronotropic action. The "total refractoriness" of the splanchnics gradually gave way to a partial responsiveness to chemoreceptor stimulation during the second hypotensive period. Increase of splanchnic outflow could then be obtained, with latent periods greatly exceeding the normal ones.

In summary, arterial stretch receptors (A fibers), were not responsible for the initial blood pressure drop and it must be assumed that vagal fibers originating from coronary chemoreceptors (?) form the afferent limb of this reflex. The secondary, long lasting hypotension, however, was probably sustained by the continuous pressoreceptor discharge, since it was present in vagotomized animals with carotid nerves intact. The alteration of the splanchnic outflow, was probably then the resultant of the continuous pressoreceptor discharge as recorded in figure 7.

Protoveratrine A and B had approximately the same latency, effectiveness and duration of hypotension in our experiments; it should be said, however, that we very rarely used the alkaloids more than once in the same animal, and had to make comparison on different preparations. The reason was that the continuous discharge of pressoreceptors remained unchanged for long periods of time, even in the face of marked intraarterial pressure changes or post mortem. Administration of calcium chloride (CaCl₂) decreased or abolished the rhythmic pressoreceptor discharge, including instances where it existed before protoveratrine was given. Although the blood pressure was increased by CaCl₂, the splanchnic activity was uniformly further decreased, indicating a peripheral (postganglionic?) or cardiac action of CaCl₂. Protoveratrine administered after CaCl₂ was not followed by renewed hypotension, both because the initiation of the rhythmic discharge was inhibited by calcium ions and because the results of an eventual repetitive pressoreceptor discharge would be ineffective in the presence of a depressed sympathetic activity.

2. Serotonin (5-hydroxytryptamine). (Serotonin) 5-hydroxytryptamine creatinine sulfate was administered intravenously in doses of 5 to 50 µg per Kg. Following a transient blood pressure rise, hypotension occurred abruptly, within 10 seconds after the start of intravenous injection. A considerable bradycardia was present during the major part of the hypotensive period. Recovery was relatively quick after about one minute, and with moderate doses of serotonin (not exceeding 20 µg per
Kg.) the gradual return to control blood pressure levels was complete within 5 minutes. The depth and duration of hypotension in response to a given dose of serotonin varied considerably from one animal to another, but the response of each animal was constant if the doses were not exceptionally large and were given at least 30 minutes apart.

The activity of the carotid sinus and splanchnic nerves displayed the following changes (fig. 8): during the initial blood pressure rise, the presso-receptor activity was enhanced in accordance with the level of blood pressure and a moderate inhibition of splanchnic efferent discharges occurred; as the blood pressure returned to control levels, splanchnic activity reappeared for 2 to 4 beats, until the onset of a complete splanchnic inhibition and significant bradycardia accompanying the rapid blood pressure fall. Presso-receptor activity decreased, along with the fall in blood pressure. In addition, various disturbances of the cardiac rhythm and conduction frequently became obvious at this stage. As with protoveratrine, splanchnic activity could not be aroused with chemoreceptor excitation during this stage. During recovery from hypotension the splanchnic activity reappeared but partially recovered splanchnic discharges could easily be inhibited. Figure 9 shows such an example, where the return to normal sinus rhythm and rise of blood pressure led to immediate disappearance of the splanchnic activity, which had previously returned almost to normal level.

A moderate but constant carotid chemoreceptor excitation was observed during recovery from hypotension, contributing to the recovery of the splanchnic activity. Repeated injections of serotonin at frequent intervals (5 to 10 minutes) led rapidly to intense chemoreceptor excitation and "tachyphylaxis", and eventually pure pressor responses were obtained. Ganglionic blocking agents, even in moderately hypotensive doses (one μg per Kg. hexamethonium intravenously) completely reversed the serotonin depressor response, and a pressor effect together with
some increase in splanchnic activity was obtained. Vagotomy also reversed serotonin hypotension in the cat.

S. Adenosine Triphosphate (ATP). Intravenous injection of 100 to 1000 µg of disodium ATP also produced the typical early hypotensive and bradycardic effects seen with serotonin and protoveratrine. Hypotension after ATP was shorter than that with equally depressor doses of the other drugs, but otherwise the actions of ATP and serotonin were essentially the same, so far as latency and depressor action were concerned. Initial hypotensive levels were roughly equal after the injection of 500 µg per Kg. disodium ATP, 30 µg per Kg. serotonin creatinine sulfate or 5 µg per Kg. protoveratrine (A or B) maleate.

In spite of this superficial similarity of the blood pressure and heart rate responses, the neurograms after ATP disclosed significant differences. Pressoreceptor activity decreased as hypotension progressed, but in contrast to serotonin, the splanchnic activity increased significantly and remained so throughout the hypotensive period (fig. 10). Vagotomy did not diminish the duration of ATP hypotension, but the depth of the hypotension was decreased, probably because of the ensuing tachycardia.

Intracarotid injections of stimulants into the "contralateral" carotid area elicited further strong splanchnic discharges. In the one instance where the initial ATP hypotension was not accompanied by increased splanchnic outflow, the intracarotid injection of sodium cyanide immediately elicited a strong splanchnic discharge.

Chemoreceptor excitation has been described after intracarotid injections of large amounts of ATP and we were able to confirm this with the doses employed above intravenously. However, the onset of the increased activity was delayed and occurred mainly during the period of recovery from the hypotension.

**Discussion**

The purpose of this investigation was to correlate directly splanchnic efferent with afferent activity from the carotid and aortic receptor areas. Normally, splanchnic discharge was maximal at the end of each pressoreceptor burst, but a slow shift from pulse to pulse was obvious; this indicates that other factors, besides inhibitory inflow from arterial stretch receptors, affect the splanchnic centers; in acute hypotension, however, the enhanced splanchnic discharge had a fixed relation to pressoreceptor activity within each cardiac cycle, different from animal to animal. Under these conditions, the factors responsible for the normal slow shift seem ineffective, probably on account of a stronger excitatory drive to the splanchnic centers. Each splanchnic burst, however, is conditioned by its corresponding pressoreceptor burst, as shown by the immediate inhibitory effects of rapid rises in intrasinusal or systemic blood pressure (figs. 4 and 9).

Our results are in general accord with the conclusion of Gernandt and Zotterman that there is no physiological or pharmacological evidence supporting the existence of vasodilator fibers in the major splanchnics. Splanchnic activity was found to increase whenever an increased "vasoconstrictor tone" should be expected and vice versa.

Intravenously injected protoveratrine, serotonin and ATP induced a rapid hypotension and bradycardia, both dependent (excepting with ATP to some extent) upon the intactness of the vagi, thus confirming findings by previous numerous investigators. The latent period of 5 to 10 seconds is compatible with that obtained by intracoronary injection of veratridine or ATP, both supposedly activating the afferents of the v. Bezold-Jarisch reflex.

So far as the initial depressor response to these three drugs is concerned, it can be ascertained that it is not due to stimulation of pressoreceptors, as carotid pressoreceptor activity decreased during this hypotension; thus, the vagal afferents from the heart must form the afferent limb of this reflex arc, especially for the immediate response to protoveratrine and serotonin, which is absent after vagotomy.

*Since this paper has gone to press, the results of Cannon and coworkers have come to our attention; these authors, by a somewhat different technic, have been unable, also, to demonstrate vasodilators in the cardiac sympathetics (Arch. f. d. ges. Physiol. 260: 116, 1954).
At this early hypotensive stage, protoveratrine or serotonin hypotension was associated with a profound inhibition of the splanchnics, and complete refractoriness of the latter to chemoreceptor stimulation. In contrast, hypotension from ATP was accompanied by a markedly enhanced splanchnic outflow, resembling that observed with peripheral vasodilators; chemoreceptor stimulation also led to a further enhancement of the splanchnic activity. One might argue that the increased splanchnic activity after ATP was due to stimulation of gut-inhibitory rather than vasoconstrictor fibers; both ATP and serotonin, however, promote intestinal peristalsis and this common property together with the diametrically opposite behavior of the splanchnics suggests that the activity induced by ATP is not due to stimulation of gut-inhibitory fibers. The enhancement of this discharge by chemoreceptor stimulation, followed by a blood pressure rise, is further indication of its vasoconstrictor nature.

It seems therefore advisable to differentiate, among the agents exciting the "coronary chemoreflex" (the v.Bezold-Jarisch effect) are those which do so by inhibition of cardioaccelerator and vasoconstrictor efferent outflow, and those which do not induce this second action. Inhibition of vasoconstrictors should be added to the criteria previously formulated by Dawes and Comroe (that is, hypotension and bradycardia abolished by vagotomy, very short latency, and minimal dose required upon injection into the coronary arteries). The well-known decrease of peripheral vascular resistance after ATP should therefore be ascribed to a direct vascular action and not to an inhibition of vasoconstrictors.

Protoveratrine and serotonin, on the other hand, behave quite differently during the early hypotensive stage, and their inclusion in the list of substances exciting the coronary chemoreflex seems to be well founded.

The second hypotensive period after protoveratrine, which also occurs in the vagotomized animal, is associated with pressoreceptor activation. At this point our findings disagree with those of Jarisch et al., who did not find pressoreceptor sensitization after systemic injection of "Veratrine." This difference could be due to the different alkaloidal composition of Veratrine.

Splanchnic inhibition is never complete during the second period of protoveratrine hypotension, and the reappearance of activity indicates that the coronary chemoreflex is short lasting or that the vasoconstrictors escape from inhibition. A low grade continuous activity replaces the highly synchronized normal pattern during the remaining period of prolonged hypotension. Protoveratrine does not possess ganglionic blocking action and it is safe to assume that the hypotension at this stage is directly related to the disturbed preganglionic pattern. Thus, lack of synchronization leads to decreased effectiveness of vasoconstrictor outflow, which can be overcome however, when splanchnic activity is greatly increased, as during anoxia.

The above findings suggest that the delayed action of protoveratrine seen in the human being is probably the result of pressoreceptor activation and not a v.Bezold-Jarisch effect. The long latent period, the gradual hypotension and the preservation of the vasomotor responses to cold, are comparable to the second hypotensive period of the protoveratrinized cat, during which continuous pressoreceptor discharge is associated with desynchronized splanchnic outflow.

Chemoreceptor excitation during the active phase of a v.Bezold-Jarisch effect was shown to potentiate bradycardia and may deepen incomplete splanchnic inhibition; this suggests that the central articulation of the chemoreceptors must occur with depressor and cardioaccelerator centers, as well as with their well known excitatory effect on vasoconstrictors. Thus, chemoreceptors stimulate both centers, but under normal conditions only excitation of vasoconstrictors becomes obvious, because of their higher "tone". When vasoconstrictors are unresponsive to chemoreceptor stimulation, during a v.Bezold-Jarisch effect, the response of the decelerators can be observed. Such a central articulation, as illustrated in Schaefer's schema of the central vasomotor organization, would best account for the non-specific excitatory action of chemoreceptors, which may
stimulate either pressor or depressor centers, depending upon the degree of activity of such centers at any given moment.

**Summary**

A study was undertaken, correlating pre-ganglionic splanchnic outflow with pressor and chemoreceptor inflow from the carotid sinus and aortic arch areas of the cat.

The normal splanchnic activity appears as groups of slowly conducted impulses at the end of each carotid pressoreceptor discharge; continuous activity has been found less frequently.

Splanchnic activity was enhanced by a decrease of pressoreceptor inflow or chemoreceptor stimulation. Decrease of splanchnic activity was obtained by increase of pressoreceptor or decrease of chemoreceptor inflow.

Protoveratrine (A or B), serotonin and ATP given intravenously to animals intact or with sectioned carotid nerves led to an abrupt hypotension and bradycardia (latent period 5 to 10 seconds). Recovery was fastest with ATP and slowest with protoveratrine. Vagotomy reversed the serotonin response and delayed the appearance of protoveratrine hypotension and vagotomy plus section of carotid nerves reversed that of protoveratrine also.

Changes in the electrical activity of the nerves under study were:

1. The initial hypotension from all three drugs was associated with a progressive decrease of pressoreceptor spikes.

2. Splanchnic activity was greatly decreased or abolished during this stage with serotonin and protoveratrine, but considerably increased by ATP.

3. Chemoreceptor stimulation during the early phase of protoveratrine or serotonin hypotension was totally ineffective in arousing splanchnic activity and even potentiated the coexisting bradycardia.

4. Only during recovery from protoveratrine did pressoreceptors discharge continuously and weakly synchronized splanchnic activity become obvious.

It is suggested that protoveratrine and serotonin, but not ATP should be included in the family of drugs causing a coronary chemoreflex.

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doi: 10.1161/01.RES.3.4.363

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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