Ability of Vasoconstrictor Drugs to Cause Adrenal Medullary Discharge after "Sensitization" by Ganglion Stimulating Agents

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Haaas and Goldblatt1 reported that infusion of DMPP (1,1-dimethyl-4-phenyl-piperazinium iodide), a ganglion stimulating agent,2 causes increase of pressor responsiveness to angiotensin. The experiments reported here reveal that, instead of enhancement of the direct effect of angiotensin on the cardiovascular system, the larger responses that follow treatment with DMPP are due to adrenal discharge of pressor material that is presumably composed of catechol amines. Other ganglion stimulating agents "sensitize" the adrenal in the same manner, and a variety of vasoconstrictor drugs elicit discharge from the sensitized gland.

Methods

Adult mongrel dogs weighing 8 to 16 Kg. were anesthetized with morphine (2 mg./Kg. S.C.) followed by sodium pentobarbital (15 mg./Kg. I.V.). Both vagus-sympathetic-depressor trunks were cut in 49 of 63 dogs. Intermittent positive-pressure respiration was employed in all experiments. Mean systemic arterial pressure was recorded on a smoked paper by a mercury manometer connected by heparin-filled tubing to a cannulated femoral artery. Test drugs were injected through a catheter inserted into a femoral vein; ganglion stimulating agents were infused into the other femoral vein. Angiotensin employed was the pure aspartyl1 isoleucyl1 octapeptide synthesized by Dr. F. M. Bumps.

Hind-limb perfusion experiments employed a constant output pump with electronically controlled constant pressure input reservoir which perfused the limb with the dog's own heparinized blood taken from a cannulated carotid artery. Perfusion pressure was recorded simultaneously with systemic pressure and reflected resistance to flow.

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Received for publication June 20, 1961.
initial dosage of DMPP, or with supplemental administration, the secondary response could be elicited during experiments lasting several hours. A reliable method of administering DMPP to obtain responses during prolonged experiments was to inject 10 to 12 mg. subcutaneously and to follow this with a continuous intravenous infusion of 20 to 100 \( \mu g \) / min. When injections of angiotensin were repeated at brief intervals, the secondary response became progressively smaller; when injections were discontinued, the secondary response returned to near the original value after an average of 15 to 20 minutes.

All other pressor drugs tested (norepinephrine, 2 to 10 \( \mu g \) base; renin, amount causing an average 32 mm. Hg rise in pressure; serotonin creatinine sulfate, 60 to 135 \( \mu g \); vasopressin, 0.5 to 2.0 units; phenylephrine HCl, 5 \( \mu g \); metaraminol bitartrate, 50 \( \mu g \); barium chloride, 5 mg.) also elicited a secondary response after treatment with DMPP but, on the basis of initial direct pressor activity, angiotensin was the most effective. On the other hand, in the eight dogs tested, pressor responses to occlusion of the common carotid arteries after vagus section were not followed by a secondary response though injection of angiotensin produced the response. Electrical stimulation of the cut peripheral end of a splanchnic nerve (1.5- to 3.0-volt square wave of 2-msec. duration at 5 to 6 cycle/sec. for 30 seconds) caused rise in pressure before and after treatment with DMPP. The direct response to stimulation was somewhat augmented by treatment with DMPP but was followed by a secondary response in only two of six dogs, and the response was smaller than that produced by angiotensin which was effective in all experiments.

The secondary pressor response to angiotensin after treatment with DMPP was not prevented by vagus section, atropine (2.4 mg. I.V.), ergotamine tartrate (0.25 mg. I.V., which reversed the pressor action of serotonin), section of the spinal cord at C6 one day prior to the experiments, section of the splanchnic nerves, splenectomy, intracisternal injection of cocaine HCl (100 mg.), or establishment of tachyphylaxis to vasopressin. A typical secondary response followed treatment with DMPP in one dog with chronic hypertension due to cellophane perinephritis.

Measurement of sympathetic efferent electrical activity in a renal or splanchnic nerve of three dogs revealed reflex diminution or
disappearance of activity during the secondary response, indicating that it is not caused by sympathetic vasomotor discharge.

The response was eliminated completely by tetraethylammonium chloride (TEAC, 2 to 10 mg./Kg. I.V.), as shown in figure 1, despite potentiation of the initial pressor response to angiotensin and norepinephrine. Phentolamine (0.5 mg./Kg.) also prevented the secondary response. Importantly, acute or chronic bilateral adrenalectomy completely prevented the response.

**Effect of Vasoconstrictor Drugs Given Directly into an Adrenal Artery After Systemic Administration of DMPP**

Since adrenalectomy prevented the secondary response, it was assumed that it was due to adrenal medullary discharge. Because pressor responses due to occlusion of the common carotid arteries did not elicit a secondary response, it was further assumed that drug-evoked secondary responses depended upon a direct—probably vasoconstrictor—action in, or near, the adrenal gland. Accordingly, the effect of very small dosage of angiotensin (0.04 to 0.08 μg.) given directly into a lumboadrenal artery was measured before and after systemic administration of DMPP. Intravenous injection of angiotensin did not affect systemic arterial pressure prior to treatment with DMPP but, after treatment, caused a large rise ranging from 50 to 150 mm. Hg in all of 15 dogs.

**Effect of DMPP Given Directly into Adrenal Arterial Blood Supply**

The possibility of sensitizing by infusing DMPP in much smaller amounts directly into a lumboadrenal artery was tested next. Infused into 10 dogs at a rate of 5 to 15 μg./min. until 0.5 to 2.0 mg. had been given, DMPP produced no appreciable rise in arterial pressure but had the same effect on response to angiotensin as the larger dosages given systemically. Large rises in arterial pressure were produced by angiotensin given either into the same adrenal artery or, in larger dosage, intravenously. The amounts of DMPP given directly into an adrenal artery were too small to elicit sensitization when given intravenously.

Small dosages of norepinephrine (0.25 to 0.5 μg.), serotonin (14 μg.), barium chloride (0.5 mg.), and hypertonic glucose (0.2 ml. of a 30 per cent solution) all elicited a secondary response when given into an adrenal artery after local sensitization by DMPP. These dosages were too small to elicit a secondary response when given intravenously and did not affect arterial pressure when given into an adrenal artery prior to infusion of DMPP.

In other experiments, DMPP was infused directly into the arterial blood supply of the pump-perfused hind limb at a rate of 40 to 90 μg./min. until as much as 5 mg. had been given. Vasoconstrictor responses to intrarterial injections of angiotensin (0.1 to 0.4 μg.) and norepinephrine (0.2 to 1.0 μg.) during and following the infusion were unchanged from control responses in both denervated (two experiments) and innervated (three experiments) legs. When larger amounts of DMPP were given systemically, the secondary response to intravenous injection of angiotensin was always accompanied by marked rise of perfusion pressure whether the leg was denervated or innervated, indicating the presence of circulating vasoconstrictor substance or substances.

**Effect of Other Ganglion Stimulating Agents**

Nicotine (9 μg./min.; total dose 200 μg.) and tetramethylammonium chloride (18 μg./min.; total dosage 1.1 mg.) had the same sensitizing action as DMPP when infused into an adrenal artery (fig. 2), but the number of experiments is not large enough to compare relative effectiveness on a weight basis.

**Concerning the Mechanism by Which Vasoconstrictor Drugs Cause Adrenal Discharge After Sensitization by Ganglion Stimulating Agents**

Prolonged (30 minutes to 2½ hours) ganglion stimulation induced by electrical stimulation of the thoracic or lumbar sympathetic trunk or of a splanchnic nerve (1.5- to 3.0-volt square wave of 2-msec. duration at 5 to
Secondary responses to angiotensin (A; 0.04 μg.) and norepinephrine (N; 0.25 μg.) injected into lumboadrenal artery after infusion of nicotine and tetramethylammonium chloride (TMAC) into same artery of 12.3-Kg. dog. Same dosages of angiotensin (I.V./A) and norepinephrine (I.V./N) given intravenously did not elicit secondary response. S=0.2-ml. physiological saline injected into adrenal artery. Time marks: one minute.

6 cycles/sec.) did not reproduce the sensitizing action of DMPP, though the direct response to angiotensin given intravenously was slightly augmented in some experiments. Prolonged infusion of angiotensin (0.02 to 0.4 μg./min., 1.2 to 40 μg.), norepinephrine (0.5 to 1.3 μg./min., 27 to 80 μg.), dimethylpiperidine (0.4 mg./min., 18 mg.) into an adrenal artery failed to cause sensitization. Additionally, intravenous infusion of angiotensin (0.05 to 0.8 μg./min., 5 to 80 μg.), dimethylpiperidine (0.7 mg./min., 114 mg.), N-methyl-beta-carbohydrazidopiperidine (3.6 mg./min., 135 mg.), or piperazine hexahydrate (17 mg./min., 360 mg.) also failed to cause sensitization.

Secondary responses to angiotensin and other vasoconstrictor drugs given into an adrenal artery were blocked for prolonged periods by as little as 1 mg. of TRAC given into the same artery, and this blocking action was opposed by intra-arterial injection of 0.01 mg. of prostigmine (fig. 3). Small doses of phentolamine (0.5 mg.) given into the adrenal artery also prevented the secondary responses; this dosage given intravenously did not prevent the response and did not modify response to norepinephrine given intravenously.

Since the secondary response was elicited by a variety of vasoconstrictor agents, the possible opposing effect of a vasodilator drug, sodium nitroprusside, was measured. After sensitization by DMPP, infusion of nitroprusside into an adrenal artery (10 to 20 μg./mm.) prevented the secondary response to angiotensin given either into the adrenal artery or intravenously (fig. 4.). The secondary response to angiotensin reappeared within 15 minutes after discontinuing the infusion of nitroprusside.

To determine if a mechanically induced reduction of adrenal blood supply would cause a secondary response, a lumboadrenal artery was partially constricted proximal to the adrenal for from three to five minutes before and after sensitization by DMPP given intravenously or into the lumboadrenal artery. Prior to sensitization, constriction of the artery did not have a significant effect...
Effects of TEAC and prostigmine given into adrenal artery on secondary response to angiotensin (A; 0.04 µg.) injected into same artery of 13-Kg. dog. Same dosage of angiotensin given intravenously (I.V./A) or 0.2 ml. of physiological saline (C) given into adrenal artery did not elicit secondary response. Thirty-minute interval between records; time marks: one minute.

Sensitization of the adrenal with DMPP also caused injection of potassium cyanide (0.5 mg.) into the adrenal artery to produce a marked secondary response; the same dose given intravenously, or intra-arterially before sensitization by DMPP, caused but minor change in arterial pressure. Secondary responses to occlusion of an adrenal artery and to injection of potassium cyanide were both prevented by injection of TEAC (2 to 10 mg.) into the same artery or intravenously (5 mg./Kg.) (fig. 5).

Chenoweth et al. reported that asphyxia causes a “secondary” response when preceded by prolonged infusion of norepinephrine, and they attributed the response to release of vasopressin. This observation was confirmed, and the mechanism of the secondary response was found to be different from the one accounting for the secondary response described here. Angiotensin given during infusion of norepinephrine (3 to 40 µg./min.; total dose 50 to 800 µg.) did not produce a secondary response; asphyxia did. The secondary response to asphyxia after infusion of norepinephrine was not prevented by TEAC; as noted above, TEAC eliminated the secondary response to angiotensin after administration of DMPP. The suggestion of Chenoweth et al. that the secondary response to asphyxia is due to release of vasopressin is supported by experiments here in which tachyphylaxis to vasopressin was produced. As tachyphylaxis appeared, the secondary response to asphyxia disappeared. Tachyphylaxis to vasopressin did not modify the secondary response to angiotensin after sensitization by DMPP.

In an effort to produce sustained adrenal discharge and enduring “secondary” hypertension, dosage of angiotensin (0.02 to 0.2 µg./min.) that caused no rise in pressure, or but a slight one, before treatment with DMPP, was infused intravenously after sen-
sitation by DMPP. Instead of a sustained hypertension there were wide swings in arterial pressure, each rise (50 to 120 mm. Hg) lasting 10 to 20 minutes and reappearing after 2 to 10 minutes, apparently due to periodic adrenal discharge. The spleen, observed through an abdominal incision, contracted strongly during each rise in arterial pressure.

Discussion

Ganglion stimulating agents cause a striking quantitative change in response of the adrenal medulla to agents, or procedures, that interfere with normal oxygenation or arterial blood supply. It was shown by Büllbring, Burn, and de Elio that decreased oxygen supply, whether produced by lowered arterial oxygen saturation or diminished blood supply, causes discharge of epinephrine from the dog's perfused adrenal gland. The experiments reported here reveal that this response is enhanced greatly by administration of a ganglion stimulating agent in dosage that by itself does not cause appreciable discharge. Injection of vasoconstrictor drugs or potassium cyanide into an adrenal artery in dosages that did not affect arterial pressure, or brief constriction of the artery which also had no effect on systemic pressure, all caused large and prolonged rises in arterial pressure after administration of a ganglion stimulating agent. This adrenal response thus appears to be mediated by a local neural mechanism that apparently includes both a cholinergic synapse and an adrenergic receptor. The cholinergic synapse is presumably ganglionic, since the response was prevented for prolonged periods by injection of very small amounts of TEAC into the adrenal artery; blockade was opposed by injection of prostigmine. Atropine did not modify the response. The adrenergic component of the mechanism causing adrenal discharge was blocked by injection of minute dosage of phentolamine into the adrenal artery; this dosage of phentolamine did not modify systemic response to intravenous injection of norepinephrine.

Figure 5

Effect of DMPP and TEAC on responses to partial constriction of adrenal artery (Art. cons.) and to KCN (0.5 mg.), angiotensin (A; 0.04 μg.), and norepinephrine (N; 0.5 μg.) given into adrenal artery of 11.2-Kg. dog. Sixty-minute interval between first and second and 45-minute interval between second and third records; time marks: one minute.
It is well established that a variety of centrally mediated neurogenic reflexes cause catechol amine discharge from the adrenal medulla (see von Euler\textsuperscript{6} for review). However, judging from the effect on systemic pressure, catechol amine discharge resulting from centrally mediated stimuli is rarely, if ever, as large as that due to local stimulation after treatment with a ganglion stimulating agent. It is suggested that the local neural mechanism causing release of catecholamines is activated by receptors located in, or near, the adrenal that are sensitive to change in oxygen tension. It is suggested further that these hypothetical chemoreceptors are largely independent of central control since, following sensitization, the usual large adrenal discharge did not follow occlusion of the common carotid arteries. The infrequent small discharge that followed electrical stimulation of the splanchnic nerve may have depended upon an indirect effect of circulating catechol amines. Alternatively, but less likely, failure of the sensitized gland to respond vigorously to neurogenic stimuli of central origin may have been due to a blockade at some point by the ganglion stimulating drug.

Piperidine has been shown by von Euler\textsuperscript{7} to be present in human urine, and piperidine and its propyl derivative, coniine, have been shown by Koppanyi\textsuperscript{8} to have a ganglion stimulating action that is followed by a nicotine-like paralysis. While infusion of several piperidine and piperazine derivatives other than DMPP in a limited number of experiments did not cause sensitization, the possibility exists that normally occurring compounds of this type may influence the sensitivity of receptor mechanisms.

Among the various vasoconstrictor drugs tested, angiotensin, on the basis of pressor activity, was the most effective in eliciting discharge from the sensitized gland. It is not now known whether the recently demonstrated ability of angiotensin to cause liberation of aldosterone\textsuperscript{9} from the adrenal gland may also be enhanced by administration of ganglion stimulating or similar drugs.

Summary

Administration of 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) subcutaneously, intravenously or into a lumboadrenal artery for 20, or more, minutes in dosages that caused little, or no, change in arterial pressure caused the adrenal glands to respond to intravenous or local intra-arterial injection of vasoconstrictor drugs (angiotensin, norepinephrine, serotonin, vasopressin, phenylephrine, metaraminol, or barium chloride) by discharging large amounts of pressor material. Nicotine and tetramethylammonium chloride given into a lumboadrenal artery had the same sensitizing effect; prolonged electrical stimulation of the cut peripheral end of the thoracic or lumbar sympathetic trunk or splanchnic nerve did not. Sensitization persisted during infusions or for at least one hour after subcutaneous administration of a ganglion stimulating agent. As after intravenous injection of a pressor drug, adrenal discharge caused reflex decrease of efferent sympathetic nerve activity. Following sensitization, partial constriction of a lumboadrenal artery proximal to the adrenal, or injection of potassium cyanide into the artery, produced responses comparable to those elicited by injection of vasoconstrictor drugs; carotid occlusion or electrical stimulation of the cut peripheral end of a splanchnic nerve did not cause the same large adrenal discharge. Partial constriction of the adrenal artery, or drug injection, did not cause adrenal discharge prior to administration of a ganglion stimulating agent. Discharge due to intra-arterial injection of a vasoconstrictor drug was prevented by simultaneous infusion of a vasodilator drug, sodium nitroprusside, into the adrenal artery. The sensitizing effect of the ganglion stimulating drugs was not prevented by atropine, ergotamine, spinal cord section at C6, splanchnic nerve section, or establishment of tachyphylaxis to vasopressin. It was eliminated for prolonged periods by injection of very small dosage of tetraethylammonium chloride or phentolamine into the adrenal artery. It is concluded tentatively that ganglion stimulating agents cause sensitization.
of receptors in, or near, the adrenal glands which, through a local neural mechanism containing a cholinergic synapse and an adrenergic receptor, respond to hypoxia by causing discharge of catechol amines.

References


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Beginning January, 1962

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Circ Res. 1961;9:1247-1253
doi: 10.1161/01.RES.9.6.1247

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

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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