Sympathetic Ganglionic Transmission and the Cardiovascular Changes of the Defense Reaction in the Cat

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ABSTRACT

The effects of ganglionic blockade on the circulatory components of the defense reaction were studied in 25 anesthetized cats. Hexamethonium alone partially blocked the response; a combination of hexamethonium and atropine was necessary to block it completely. Vasodilation of skeletal muscle was reversed to vasoconstriction during hexamethonium infusion. Direct recording from pre- and postganglionic sympathetic nerves showed persisting ganglionic transmission during hexamethonium infusion that was subsequently blocked by atropine. Unit recordings showed that some ganglion cells were completely blocked by hexamethonium alone. Preganglionic discharge was unaffected. It is suggested that ganglion cells giving rise to cholinergic vasodilator fibers have fewer atropine-sensitive receptor sites than do cells from which adrenergic constrictor fibers arise.

ADDITIONAL KEY WORDS

inhibitory postsynaptic potential
excitatory postsynaptic potential
hypothalamus phenoxybenzamine
vasodilation skeletal muscle hexamethonium
electroneurogram atropine

Preganglionic stimulation of sympathetic ganglion cells elicits an excitatory postsynaptic potential which is blocked by hexamethonium (1). Two slower responses also occur, an inhibitory postsynaptic potential and a slow excitatory postsynaptic potential (1-4). The slow responses are distinguished from the fast excitatory postsynaptic potential by very long synaptic delays (hundreds of milliseconds instead of 1 msec) and durations of many seconds instead of milliseconds (2). The slow excitatory postsynaptic potential is not affected by hexamethonium but is blocked by atropine (2, 5, 6). The slow inhibitory postsynaptic potential may be mediated by an interneuron and is diminished by dibenzylene, propranolol, or atropine (7).

Repetitive supramaximal stimulation of the preganglionic thoracic sympathetic nerve elicited synchronous activation of stellate ganglion cells without after-discharge (8). This caused both tachycardia and a rise in arterial blood pressure. During infusion of hexamethonium, an asynchronous discharge was provoked and persisted for 60 to 90 seconds; it caused a similar but smaller circulatory response. The asynchronous discharge arose from the late excitatory postsynaptic potential and was blocked by atropine (2, 6, 8).

It was also found that the circulatory components of the defense reaction elicited by hypothalamic stimulation were blocked completely only by a combination of hexamethonium and atropine (9). Moreover the outstanding circulatory feature of the defense reaction, vasodilation in skeletal muscle, was reversed and vasoconstriction occurred after administration of hexamethonium; the reversed response was subsequently abolished by atropine. In the present experiments an electroneurographic analysis of the activity of pre- and postganglionic sympathetic nerves during hypothalamic stimulation was made.
Methods

The experiments were done using 25 cats (2.5 to 5.0 kg) anesthetized with a chloralose-urethane mixture (60 mg/kg chloralose, Sigma Chemical Co.; 250 mg/kg urethane) injected intravenously after ether induction. The trachea was cannulated and intermittent positive-pressure respiration was begun. The animals were paralyzed with gallamine triethiodide (Flaxedil, Davis and Geck), 2.0 mg/kg iv; these doses do not interfere with sympathetic ganglionic transmission (8). The animals were allowed to recover from paralysis to insure that pinching the hindlimb did not provoke a pain-like reaction. The stroke of the respiration pump was adjusted to maintain the end expiratory Pco₂, measured continuously with an infrared CO₂ analyzer at about 35 mm Hg which is normal for man in Salt Lake City (elevation 4,500 ft). The arterial oxygen saturation measured by the Van Slyke method varied between 92% and 95%. Rectal temperature was maintained at 37°C by placing each cat on an electric heating pad.

Arterial blood pressure was registered by a strain gauge connected to a polyethylene catheter which had been inserted into a femoral artery and passed retrograde to the aorta. Venous pressure was measured with a strain gauge connected to a polyethylene catheter that had been passed through an external jugular vein to the region of the right atrium. Each catheter-manometer system had a flat (±5%) frequency response of 20 cps (10). The pressure pulse signal was passed through an RC network to obtain mean pressure.

The left forelimb was skinned from axilla to ankle and the paw circulation occluded at the ankle by a tight ligature. The muscles were covered with warm, saline-soaked gauze, and the skin was sutured over them. An electromagnetic flow transducer (diameter 1.0 mm), connected to a Statham Module M-4001 amplifier, was placed around the axillary artery; zero flow was obtained by occluding the artery distal to the transducer, and in-vitro calibration, using steady flow and blood or saline, was done at the end of each of 18 experiments. The reliability of such calibrations, in which the position of the transducer relative to the vessel was undoubtedly different from that in vivo, is unknown; however, such calibrations gave flow values similar to those obtained by Eliasson et al. (11), who used a drop recorder.

Stainless steel microelectrodes (tip diameter 2 to 5μ) were made according to the method of Bishop and Collin (12), and insulated to the tip with Ins-X. The electrode resistance varied from 25 to 75 kilohms. The microelectrode was inserted by stereotaxic means into the perifornical region of the hypothalamus. The indifferent electrode was made the anode and placed in the frontal bone. The electrical stimuli were rectangular pulses, 2 msec in duration. The frequency of stimulation was usually 70/sec, and the duration of the train was 10 seconds. Current was monitored continuously on an oscilloscope connected across a 1-ohm series resistor; slightly suprathreshold currents (0.8 to 1.5 ma) were used. When stimulation evoked both a maximal skeletal muscle vasodilatation, blocked by atropine (0.4 mg/kg iv), and a slight rise in arterial blood pressure, it was considered that the hypothalamic integrative area for the defense reaction had been located and an optimal response obtained (11, 9). Histological confirmation had previously been obtained in another series of experiments (8).

The left stellate ganglion was exposed according to the method described by Brown (8). One electromyogram was recorded from the peripheral end of a cut preganglionic ramus (either T1 or T4); this was an index of preganglionic neuronal activity. A second electromyogram was recorded from the peripheral end of slips of nerve dissected out of the vertebral nerve; this provided an index of the activity of stellate ganglion cells innervating the forelimb (13). In 5 experiments, strands of nerve containing only 2 or 3 active fibers were dissected out of the postganglionic nerve. Platinum electrodes were used for recording. The bandwidth of the a-c coupled preamplifier was 30 to 34 kcps. Electroneuromyograms, arterial blood pressure, and skeletal muscle blood flow were recorded on a Honeywell Visicorder (Model 1508). The galvanometers used for recording the electromyograms had measured flat (±5%) frequency responses of 4.8 kcps.

Hexamethonium chloride (Hexameton, Burroughs Wellcome) was injected intravenously at a rate of 50 to 200 mg/kg/hour using a Harvard constant-infusion pump. This dose blocks the early excitatory postsynaptic potential and synchronous activation of stellate ganglion cells (8). Atropine sulfate 10 to 100 μg/kg was injected intravenously as a single dose during the hexamethonium infusion. Phenoxybenzamine (Dibenzyline, Smith, Kline and French), was injected intravenously in a dose of 1.0 mg/kg. Axillary arterial resistance was calculated as the ratio of

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\frac{\text{aortic pressure (mm Hg)}}{\text{axillary arterial flow (ml/min)}}
\]

Venous pressure was assumed to be constant.

Results

Figure 1A shows the striking increase in blood flow through forelimb skeletal muscle elicited during stimulation of the hypothalamic integrative area for the defense reaction.
The response was similar to that obtained in the hindlimb (11, 9). A small increase in pressure with no change in flow (vasoconstriction) preceded the increase in flow. Atropine prevented the vasoconstriction but the initial vasoconstriction was slightly enhanced (Fig. 1B). The peak changes of vascular resistance in forelimb skeletal muscle are given in Table 1; latency is the time from the onset of stimulation to the peak changes. A small increase of aortic blood pressure occurred in 17 out of 20 experiments (see Figs. 1A and 2A); in 3 experiments the aortic pressure fell (Fig. 3). The small rise was sustained at a somewhat lower level for 30 to 120 seconds in 12 out of 17 experiments (Fig. 1A). In 5 out of 17 experiments the small increase was succeeded by a fall below control levels and a subsequent slow recovery (Fig. 2A).

In 3 experiments, the initial peak vasoconstriction (see Table 1) was abolished by dibenzyline; the vasoconstriction was unaffected or slightly increased.

The effect of hexamethonium on the response to hypothalamic stimulation is shown in Figure 3B, and the results of 6 experiments are presented in Table 1. Before hexamethonium, the forelimb vascular resistance initially increased 5% above control and was succeeded by a fall of 70% below control. During hexamethonium infusion, the resistance during stimulation increased by 70% above the control level.

### Table 1

<table>
<thead>
<tr>
<th>Vascular Resistance in Forelimb Skeletal Muscle</th>
<th>Before hypothalamic stimulation</th>
<th>After hypothalamic stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak vasoconstrictor response</td>
<td>Latency (sec)</td>
</tr>
<tr>
<td>Group 1 Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After atropine</td>
<td>21.2 ± 3.1</td>
<td>23.8 ± 3.5</td>
</tr>
<tr>
<td>Group 2 Control</td>
<td>20.0 ± 3.3</td>
<td>24.5 ± 3.4</td>
</tr>
<tr>
<td>After hexamethonium</td>
<td>20.0 ± 1.0</td>
<td>21.0 ± 5.0</td>
</tr>
<tr>
<td></td>
<td>15.8 ± 1.5</td>
<td>26.8 ± 3.2</td>
</tr>
</tbody>
</table>

The data for group 1 were obtained from 7 experiments in 7 different cats; the data for group 2 came from 6 experiments in 6 other cats. Numbers are means ± SEM. Vascular resistance in forelimb equals (aortic pressure in mm Hg)/(axillary arterial flow in ml/min).
value, reaching its peak in about 15 seconds, and was followed by a fall to 27% below control about 30 seconds after stimulation. Thus the vasodilator response which had previously been predominant was now subsidiary to a predominantly vasoconstrictor response. The latencies to peak vasoconstrictor and vasodilator effect were markedly prolonged. The vasomotor responses were mediated by the vertebral nerve since they were prevented by its section (3 experiments before hexamethonium infusion; 3 during infusion). This is in agreement with the findings of Mizeres (13) and unpublished observations of my own which show that the sympathetic innervation of the forelimb is mediated by this nerve. The delayed vasodilation during hexamethonium infusion (Fig. 2B) persisted after nerve section although it was reduced; at present, there is no explanation for this result.

Injection of atropine during hexamethonium infusion (6 experiments) abolished the circulatory response to hypothalamic stimulation (Fig. 2C) (9). It has been shown that during hexamethonium infusion, synchronous repetitive preganglionic sympathetic volleys can elicit an asynchronous postganglionic barrage of nerve impulses after an after-discharge and that this effect can be blocked by atropine (8). In the present experiments, it might also be expected that synaptic transmission which persisted despite the infusion of hexamethonium can be blocked by atropine. This was confirmed electrophysiologically.

In 6 experiments, hypothalamic stimulation provoked a large burst of activity in the preganglionic nerve which began after a latency of 10 to 50 msec and ceased immediately when stimulation was interrupted (Fig. 3A). After a slightly longer latency the postganglionic nerve which showed phasic activity synchronous with respiration (14) also registered a large burst of activity due to synchronous discharge of ganglion cells. Phasic activity was obliterated by the now continuous burst. The discharge stopped abruptly when hypothalamic stimulation was terminated. The next expected phasic burst did not occur and subsequent bursts were smaller until the pulse pressure returned and arterial blood pressure returned to control. This inhibition was probably reflex, initiated from the sinoaortic baroreceptors. It is of interest to note that during hypothalamic stimulation, the arterial blood pressure fell while the postganglionic discharge was maximal. During Ca infusion (Fig. 3B), hypotha-
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FIGURE 3

A: From top to bottom: Electroneurogram recorded from peripheral end of T4 white ramus; arterial blood pressure; electroneurogram from peripheral end of a slip of the vertebral nerve; signal marker. During signal, hypothalamus was stimulated. B: During C6 infusion. C: After atropine. Note the progressive fall in mean arterial blood pressure between A and B, B and C. Similar response is seen in Figure 2.

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FIGURE 4


Sympathetic stimulation provoked a similar burst in the preganglionic nerve but the discharge in the postganglionic nerve was altered. The synchronous discharge of the ganglion cells previously elicited was replaced by an asynchronous discharge, hence the smaller amplitude of the spikes. Moreover, the discharge persisted for 30 to 300 seconds after hypothe-
lamic stimulation was discontinued. Similar changes have been described following direct stimulation of the preganglionic nerve trunk (8). Although the arterial blood pressure rose during stimulation, there was no inhibition of the asynchronous discharge. Following atropine, hypothalamic stimulation again provoked a similar burst in the preganglionic nerve, but all activity in the postganglionic nerve was blocked. The arterial blood pressure was now unaltered during stimulation.

In Figure 4A, the response to hypothalamic stimulation of two fibers dissected out of the vertebral nerve is shown. The discharge began and ended almost simultaneously with the hypothalamic stimulation. The frequency of the smaller spike was about 9/sec, while that of the large was about 2/sec. During C6 infusion (Fig. 4, B and C), hypothalamic stimulation no longer elicited the larger spike discharge. After a latency of about 2 seconds, the smaller spike appeared and now had a peak frequency of about 3/sec; its discharge persisted for about 60 seconds after hypothalamic stimulation ceased. Following atropine, hypothalamic stimulation could not provoke the smaller spike discharge. Similar results were obtained in 3 experiments when preganglionic stimulation was substituted for hypothalamic stimulation.

Discussion

The present experiments show for the first time that an asynchronous postganglionic sympathetic discharge which persists despite hexamethonium infusion, and is subsequently blocked by atropine, can be evoked by stimulation of the hypothalamic integrative area for the defense reaction. The results are made more meaningful since the defense reaction elicited in anesthetized animals has the same circulatory components as the response to stress in unanesthetized animals (15, 16). Moreover the prolonged after-discharge is one of the few examples of long-lasting electrical changes in the nervous system (7).

These experiments show further that not all ganglion cells can be stimulated to discharge during hexamethonium infusion (Fig. 4). Unfortunately, no statistical evidence is available because of the great technical difficulties in obtaining single unit recordings of postganglionic sympathetic nervous activity. It should also be noted that the activity in preganglionic nerves was unaffected by hexamethonium or atropine or both.

The reason for the reversal of skeletal muscle vasodilation to a predominantly vasoconstrictor response during hexamethonium infusion is unknown. It is suggested that during infusion, proportionately fewer ganglion cells from which cholinergic vasodilator fibers originate have slow excitatory postsynaptic potentials in response to hypothalamic stimulation than do ganglion cells from which adrenergic constrictor fibers arise. Other possibilities exist—for example, some ganglion cells may have large inhibitory postsynaptic potentials that prevent the slow excitatory postsynaptic potentials from generating action potentials.

References

8. BROWN, A. M.: Cardiac sympathetic adrenergic pathways in which synaptic transmission is
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Reaction in the Cat

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