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Neuronal activity of the cat supraoptic nucleus is influenced by muscle small-diameter afferent (groups III and IV) receptors

(neurosecretory neuron/muscle afferents/vasopressin/muscle contraction/exercise)

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ABSTRACT **In anesthetized cats, responses of single neurosecretory neurons of the supraoptic nucleus to activation of muscle receptors were investigated. Electrical stimulation (1–3 pulses at 200 Hz) of group III and IV pure muscle afferents (gastrocnemius nerve) evoked excitation of >50% of supraoptic nucleus neurons (n = 50), whereas stimulation of group Ia or Ib fibers was ineffective. Baroreceptor stimulation inhibited 95% of these supraoptic nucleus neurons that responded to activation of muscle afferents. Excitation of receptors in the gastrocnemius muscle by intra-arterial injection of chemicals (NaCl, KCl, and bradykinin) increased firing rates of most (84%, 74%, and 80%, respectively) neurosecretary neurons. The magnitude of the excitatory response was dose dependent—bradykinin being the most effective. The response disappeared after muscle denervation. When the gastrocnemius muscle alone was contracted phasically by ventral root stimulation, discharges of the supraoptic nucleus neurons increased, whereas quick stretch of the muscle had no effect. We conclude that activation of muscle receptors by chemical or mechanical stimulus can directly excite neurosecretory neurons in the supraoptic nucleus and that afferent impulses are carried by polymodal fibers of small diameter but not by the largest afferents (group I) from the muscle. The results may relate to increased concentrations of plasma vasopressin during exercise.**

Physical exercise is accompanied by antidiuresis and increased plasma level of vasopressin (1–7) as well as by respiratory and cardiovascular changes (8–12). It has been suggested that these autonomic responses do not result from secondary changes induced by increases in blood volume, core temperature, or metabolic rate during exercise but result, instead, from direct excitatory neural input from polymodal receptors in the muscle to the autonomic centers (10, 11). We showed previously that the neurosecretory neurons in the supraoptic nucleus (SON) of the hypothalamus were affected by excitation of peripheral somatic as well as visceral afferents (13). Thus, the increased level of plasma vasopressin could also be due to activation of polymodal receptors in striated muscle.

The present investigation was done for two purposes: (*i*) to study characteristics of afferent fibers from the muscle that excite neurosecretory neurons in the SON and (*ii*) to study the pattern of response of SON neurons to chemical or mechanical stimulation of muscle receptors. A short account of this work has been presented (14).

METHODS

Preparation. Adult cats (2.5–3.2 kg in body weight) of either sex were anesthetized with i.p. administration of α-chloralose (60 mg/kg) or sodium pentobarbital (Nembutal, 35 mg/kg). Supplemental doses of the same anesthetic agents or sodium thiopental (Pentothal, 2–5 mg/kg) were given i.v. whenever necessary. No muscle relaxant was used. A trachea cannula was inserted, and the femoral artery and vein were cannulated for blood pressure recording and fluid or drug administration. Pneumothorax and artificial ventilation was used whenever necessary to minimize movement of brain tissues. The rectal or esophageal temperature was maintained between 37–38°C by a heating pad and an overhead lamp. The animal was placed in a stereotaxic head holder and hemispherectomy was done by the method described (15). The exposed surface of the hypothalamus was constantly irrigated by warm Locke's solution, although sufficient cerebrospinal fluid was usually present.

Excitation of Muscle Receptors. Four methods were used to activate muscle receptors (Fig. 1). All stimuli were given on the side contralateral to that of neuron recording.

(*i*) *Electrical stimulation.* The gastrocnemius nerve (pure muscle nerve) was severed from its attachment to the muscle and stimulated through a bipolar platinum electrode placed on its central end. A conventional bipolar recording electrode was also placed on the nerve a few cm central to the stimulating electrode to measure the threshold and the conduction velocity of stimulated fibers. A stimulus consisted of three pulses at 200 Hz and 0.5-msec duration at 0.8 Hz. Stimulus intensities used were expressed as multiples of the lowest threshold (× T) for exciting the largest fibers in the nerve (group Ia). By monitoring afferent action potentials in each preparation, we could determine threshold values (× T_{Ia}) for group III and IV fibers. All other branches of the sciatic nerve trunk innervating the same limb were severed. Exposed muscles and nerves were covered with warm mineral oil in a pool made from the skin of the lower limb.

(*ii*) *Chemical stimulation.* Methods used by Franz and Mense (16) and Mizumura and Kumazawa (17) were used with certain modifications. Chemical agents (NaCl, KCl, and bradykinin triacetate) were injected locally through a small polyethylene cannula inserted into the sural artery (a branch of the popliteal artery). All other branches of the popliteal artery were carefully tied to prevent minor leakage, an essential procedure (16, 18). Sometimes ligation around the femur was necessary to occlude as much blood as possible, except that flow through the femoral vessels (19). After clamping the popliteal artery for 30 sec, a 0.3-ml injection of test solution was followed by 1.0 ml of Locke's solution within 20 sec. The arterial clamp was released 60 sec after the injection.

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Abbreviations: SON, supraoptic nucleus; × T_{Ia}, multiple of threshold stimulus for group Ia afferent fibers.

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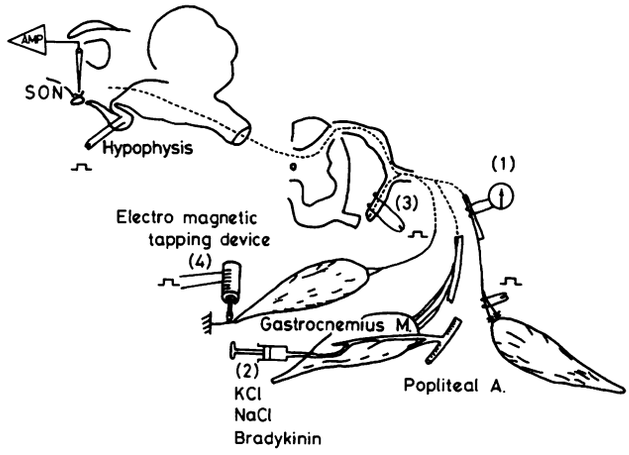

FIG. 1. Schematic of four methods used to excite muscle receptors. (*1*) Electrical stimulation of pure muscle afferents; (*2*) chemical stimulation by intra-arterial injection of KCl, NaCl, and bradykinin; (*3*) contraction of the gastrocnemius by stimulation of centrally severed ventral root; and (*4*) a quick muscle stretch by Achilles tendon tap. Recordings were made from neurons in the SON identified by stimulating their axons in the pituitary.

(*iii*) *Muscle contraction.* After laminectomy the ventral root of the 6th or the 7th lumbar segment was sectioned, separated from other roots, and placed on a bipolar stimulating electrode in the mineral oil pool. The peripheral end of the severed ventral root was stimulated to produce contractions of the gastrocnemius muscle. All branches of the sciatic nerve trunk innervating the same limb except the gastrocnemius nerve had been severed, and the femur and tibia were fixed by clamping the distal end of the bones to prevent movement. The skin surrounding the muscle had also been completely removed. A stimulus consisting of three pulses at 100 Hz, 0.1 V, and 0.1 msec was given every 1.2 sec.

(*iv*) *Muscle stretch.* To activate the group Ia afferents of muscle spindles, the Achilles tendon was tapped by a mechano-magnetic device that produced stretch at a speed of 15 mm/sec applied for 0.2 sec (see references in ref. 20). For these experiments, both femur and tibia were fixed firmly.

Recordings from the SON Neurons. Electrical activity of the hypothalamic neurons was recorded by use of conventional glass capillary electrodes (20–30 MΩ resistance) filled with 0.5 M NaCl containing 2% (wt/vol) Pontamine sky blue dye (Chicago sky blue 68, Sigma). In earlier work insl-X-coated tungsten microelectrodes were also used. A recording electrode was inserted into the SON with the aid of an operating microscope and stereotaxic map. A bipolar metal stimulating electrode was placed on the neurohypophysis. Neurosecretory neurons in the SON were identified by antidromic excitation induced by stimuli applied to the neurohypophysis (single pulses, 0.5–1.5 mA); collision techniques were also used for this purpose. Extracellularly recorded action potentials were amplified, and their numbers were counted with the use of a window discriminator. Counted spikes per 0.5–1 sec were displayed on the polygraph (Grass) together with the blood pressure and heart rate. Action potentials were also recorded and post-stimulus-time histograms were constructed from 32 to 256 recorded responses.

To activate baroreceptors, the carotid sinus was distended by a small balloon catheter introduced into the carotid sinus region through the common carotid artery at the side ipsilateral to the recording site (21).

Denervations of baroreceptors were accomplished by crushing the carotid sinus nerves bilaterally as well as the nerve fibers surrounding the carotid bifurcation and by bilateral vagotomy at the neck.

After each experiment, locations of the recording and stimulating electrodes were marked by depositing Pontamine sky blue dye or by passing current through the electrodes. The brain was fixed in Formalin, and frozen sections, 40 μM in thickness, were stained with cresyl violet and examined histologically.

RESULTS

Excitation of Muscle Receptors by Electrical Stimulation. Fifty identified neurosecretory neurons in the SON were tested by stimulating the gastrocnemius nerve. As illustrated in Fig. 2*A* trace b, at low-intensity stimulation, below threshold for group III afferents (4 × T_{Ia}) judging from afferent impulses monitored (see *Methods*), no SON neuronal response was seen. When stimulation intensity was increased to include group III afferents (10 and 20 × T_{Ia}, respectively, in Fig. 2*A* traces c, d, and e) the excitatory effect of the stimulus became clear. The latency of the

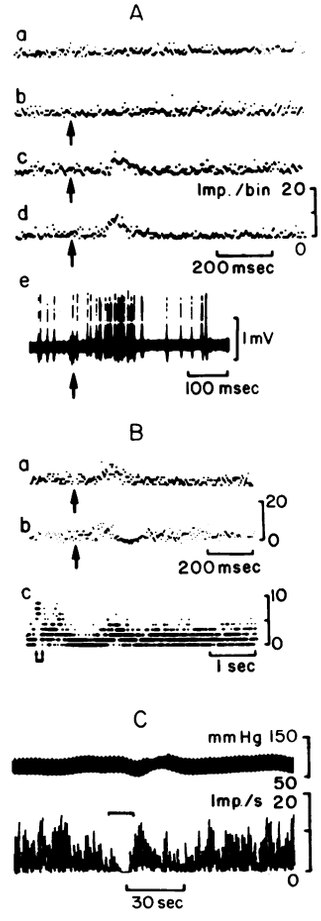

FIG. 2. Effects of stimulating pure muscle afferents of the gastrocnemius muscle on discharge frequencies of single neurosecretory neurons. (*A*) Traces a–d, post-stimulus-time histograms (bin width was 4 msec, 64 trials) after stimulation (arrows) at 0.8 Hz (three pulses at 200 Hz, 0.5 msec). Stimulus intensities were as follows: trace a, control; trace b, 4 × T_{Ia}; trace c, 10 × T_{Ia}; trace d, 20 × T_{Ia}. The response of trace d is also shown as 10 superimposed oscilloscope tracings in trace e. (*B*) Recordings from other SON neurons showing late inhibition after excitation with higher-intensity stimulation (three pulses at 200 Hz, 0.5 msec, 0.8 Hz). Trace a, 12 × T_{Ia}, 64 trials; trace b, 50 × T_{Ia}, 128 trials, and trace c from another neuron. The muscle nerve was stimulated (μ) at 50 × T_{Ia} with 20 pulses at 200 Hz at random frequencies triggered manually, 5-msec bin width, 100 trials. (*C*) Response of SON neuron to baroreceptor stimulation (marked by overbar ⌐) by inflating a balloon in the carotid sinus.

response ranged from 50 to 80 msec after beginning the stimulus, and excitation lasted ≈100 msec. As stimulus intensity was increased enough to excite unmyelinated as well as myelinated afferents in the gastrocnemius nerve, magnitude of the excitatory response was reduced, and the subsequent inhibitory phase became clearer. Fig. 2*B* shows that when only group III fibers were stimulated (12 × T_{Ia}) SON neurons showed only the excitatory response (Fig. 2*B* trace a), whereas at higher stimulation intensity to include unmyelinated fibers (50 × T_{Ia}), a brief weak excitation of the neuron was followed by long and clear inhibition (Fig. 2*B* trace b). Latency of the inhibitory response was generally 100 msec, but in a few neurons, latency was >500 msec (Fig. 2*B* trace c). Blood pressure was not altered by electrical stimulation of the gastrocnemius nerve at 0.8 Hz at any intensity used in this experiment.

Table 1 summarizes responses of all neurosecretory neurons in the SON. Our finding that no response was elicited by a stimulus <4 × T_{Ia} indicated that the largest myelinated afferents from muscle spindles (primary endings) and tendon organs did not affect SON neurons. With excitation of group II and large group III afferent fibers (intensity <10 × T_{Ia}), a small percentage of SON neurons were excited. The majority (62%) of neurosecretory neurons responded to a stimulus at 20–30 × T_{Ia}, which was strong enough to excite all group III muscle afferents but not unmyelinated fibers. The excitation of unmyelinated fibers in the gastrocnemius nerve, in addition to myelinated ones, did not increase percentages of responding neurons in the SON, but more neurons showed biphasic patterns of response—that is, excitation followed by inhibition.

Responses of some of these SON neurons to excitation of baroreceptors were also examined. Stimulation of the carotid baroreceptors strongly inhibited the basal activity of most (19/20) neurons that were excited by muscle afferent stimulation (Fig. 2*C*).

Effects of Stimulation by Chemical Agents. Excitation of muscle receptors by local injections of NaCl excited 84% (21/25), of KCl excited 74% (14/19), and of bradykinin excited 80% (16/20) of the SON neurons tested. Injections of Locke's solution of the same (0.3 ml) or larger volume had no effect. Although effective concentrations of each chemical differed, the excitatory response caused by NaCl and KCl was quite similar (Fig. 3, *A* and *B*): Latency was ≈5 sec after beginning an injection, but the response was brief, lasting for 20 sec, although its duration might have been longer had it not been interrupted by secondary inhibition (see below). Responses to bradykinin, on the other hand, had a long latency, over 15–20 sec, and excitation lasted for a longer period even when the drug was given at low concentrations (Fig. 3*C*). This seemingly slow response is characteristic of bradykinin; afferent impulses recorded from muscle afferents have an average latency of 9.9 sec (16) and 17–28 sec (18) after bradykinin application to muscle. Most neurosecretory neurons (8/9) responded to all three chemicals (NaCl, KCl, and bradykinin) (Fig. 4).

Table 1. Effect of electrical stimulation of gastrocnemius nerve on neurosecretory neuronal response

Stimulus intensity, × T_{Ia}	Cell response, no. Tested	Excitation only (%)	Excitation followed by inhibition (%)	Unresponsive (%)
4–10	13	2 (13)	1 (8)	10 (79)
10	24	5 (21)	1 (4)	18 (75)
20–30	21	11 (52)	2 (10)	8 (38)
50	29	10 (34)	7 (24)	12 (41)
100	22	7 (32)	7 (32)	8 (36)

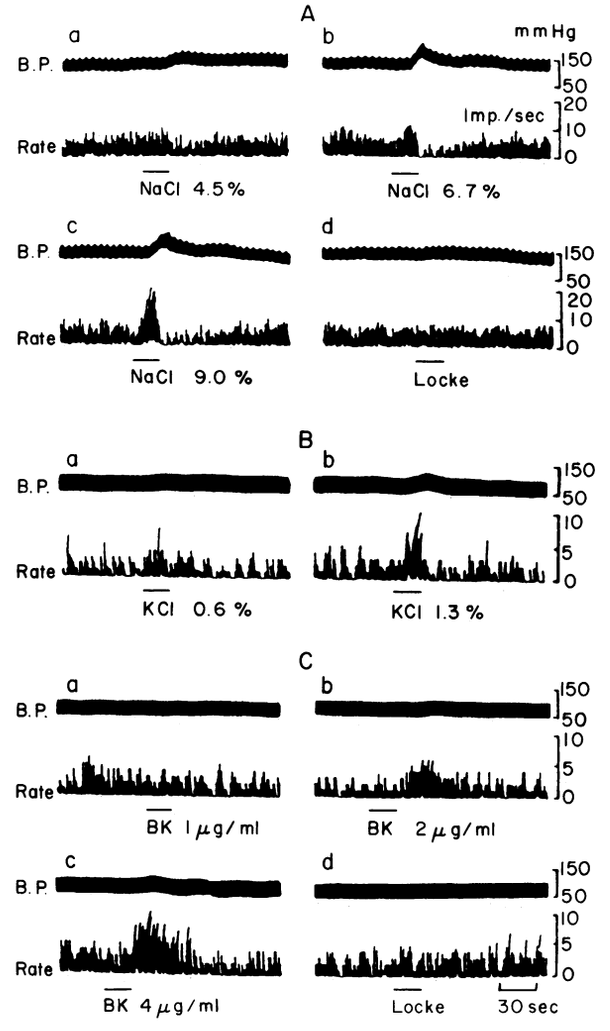

FIG. 3. Effects of chemical stimulation of the gastrocnemius muscle on SON neurons. (*A*) Different concentrations of NaCl and Locke's solution (always 0.3 ml) were injected intra-arterially at bars. Traces from top to bottom: blood pressure (B.P.), number of action potentials of single neuron counted per sec (Rate). All records are from the same neuron. (*B*) Effect of KCl injection to another cell. Injections of Locke's solution to the same neuron had no effect (data not shown). (*C*) Effects of intra-arterial injection of bradykinin (BK) on the activity of the same SON neuron (as in *B*).

The excitatory responses of neurosecretory neurons to NaCl and KCl were roughly dose dependent, and threshold doses were 1 and 0.1 M (0.3-ml injection), respectively. Threshold dose for bradykinin was 1 μM (0.3 ml), and the

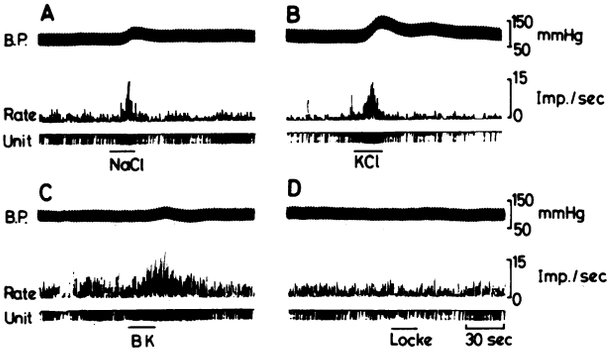

FIG. 4. Responses of SON neuron to three chemicals injected into the artery supplying the gastrocnemius. Unit-shaped pulses of neuronal discharges were directly recorded. Traces are labeled the same as for Fig. 3.

response to bradykinin reached maximum with 2 μM solution and then plateaued.

Although blood pressure was elevated after NaCl and KCl injections, the pressure began to rise after excitation of SON neurons had peaked, increasing to maximum pressure after the neuronal response was complete (Figs. 3 and 4). Because stimulation of arterial baroreceptors by increased blood pressure is known to inhibit SON neuronal activity (21, 22), elevated blood pressure after NaCl and KCl injections may cause late inhibition of SON neurons, thus shortening the excitatory period. Bradykinin injections did not evoke any strong pressor response nor any late inhibition of SON neuronal activity (Fig. 3). In three animals the same chemical stimulation was repeated after complete denervation of baroreceptors to eliminate hemodynamic changes caused by chemical injections. Results of the experiment confirmed our conclusion that the excitatory action of injected chemicals was not secondary to changes in the cardiovascular system.

In seven instances effects of injections of these chemical agents were tested after denervation of the gastrocnemius muscle. No response of SON neurons was seen, indicating that the effects produced by injected chemicals were due to activation of afferent fibers in the muscle nerve.

Effects of Mechanical Stimulation by Muscle Contraction and Stretch. To test effects of more natural stimulation of muscle receptors we evoked contraction of the gastrocnemius muscle by stimulating centrally severed lumbar (L) ventral roots (L_6 or L_7). Stimuli consisting of three pulses of 0.1 V and 0.1 msec at 100 Hz were applied to the root at a rate of one three-pulse stimulus every 1.2 sec. This stimulus strength was sufficient to produce plantar extension but not tetanic contraction. Because all nerve branches innervating hind-limb muscles except the gastrocnemius nerve were severed and the skin was removed, any effect produced by the stimulus must have been due to afferent impulses originating from the contracting muscle. Fig. 5 *A* and *B* shows that neurosecretory neurons were excited after a latency of ≈50 msec and reached peak firing rate at ≈150 msec after a short stimulus train. Similar results were obtained from seven neurons.

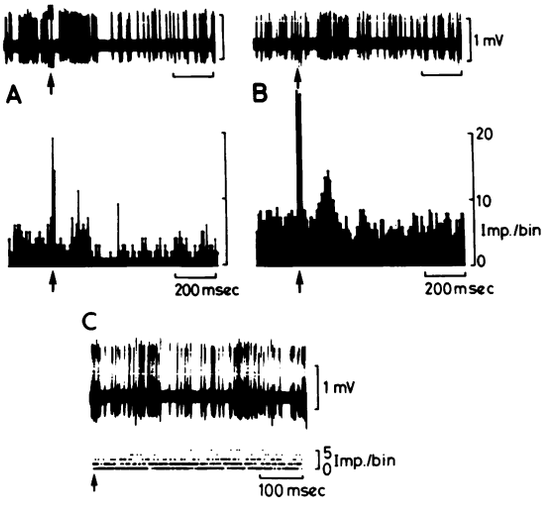

FIG. 5. (*A* and *B*) Effects of gastrocnemius muscle contraction on SON neurons. Contraction was evoked by stimulation (0.1 V for 0.1 msec, three pulses at 100 Hz) every 1.2 sec of the ventral root of the sixth (A) or seventh (*B*) lumbar segment. *A* and *B* were recorded during separate experiments. *Upper*, 10 superimposed oscilloscope sweeps; *Lower*, stimulus time histograms of 100 (*A*) and 200 (*B*) trials. Bin width was 8 msec for both *A* and *B*. (*C*) Lack of effect from tap to Achilles tendon (↟) on two SON neurons. *Upper*, 100 superimposed oscilloscope tracings; (*Lower*), stimulus time histogram, 200 trials, bin width was 2 msec. These data are from two separate experiments.

Fig. 5*C* shows the effect of short mechanical stimulus to the Achilles tendon from a mechano-magnetic device. The stimulus was such that, were ventral roots intact, it would elicit a monosynaptic tendon reflex. No effect on neurosecretory neurons was seen in all nine neurons tested, even after 100–200 superimposed responses. The finding agrees well with our results of electrical stimulation of muscle afferent fibers—i.e., excitation of the largest group of muscle afferents does not affect activity of SON neurons.

DISCUSSION

Our results clearly show that group III and IV (unmyelinated) afferent fibers from muscle cause the changes produced in SON-neuron activity, whereas excitation of the largest-diameter fibers in muscle nerves, the group Ia and Ib afferents originating from muscle spindles (primary endings) and Golgi tendon organs, do not influence the SON neurons. It is interesting that excitation of these largest afferents was also found not to cause any reflex discharge of the sympathetic (23, 24) or the vagus nerves (25), nor any respiratory and cardiovascular changes during muscle exercise (10–12).

Several investigators (16, 18, 26, 27) have presented evidence that small afferent fibers, particularly unmyelinated fibers in the muscle nerve, are excited by chemical agents, such as NaCl, KCl, bradykinin, and histamine when injected into the artery supplying the studied muscle. Some single small fibers in the muscle afferent nerves have also been shown to be excited by mechanical, thermal, and chemical stimuli (18), and these authors suggested that such polymodal fibers were probably excited by exercising muscle. Other workers found that during exercise small-diameter afferent fibers were excited by metabolites that accumulated in the blood, and these fibers caused reflex changes in cardiovascular and ventilatory systems (10, 11, 28, 29). Direct chemical stimulation of the muscle receptors also produced respiratory changes similar to those seen during exercise (17). Moreover, we showed that phasic muscle contractions caused excitation of SON neurons, a response similar to that produced by direct electrical and chemical stimulation of small muscle afferents. Thus, it is reasonable to assume that during muscle contraction and exercise, excitation of small muscle afferents can cause an increase in the activity of SON neurons as well as cause respiratory and cardiovascular changes.

Direct excitation of SON neurons by impulses from contracting muscle can occur without nociceptor activation, as indicated by observations in humans during exercise when the level of plasma vasopressin increases without any sense of pain being reported. During exercise, cardiac acceleration and a rise in blood pressure have been reported to precede any pain during muscle contraction under the condition of occluded blood supply (8, 9, 28). Coote and his associates (10, 29) stated that unmyelinated fibers from "metabolic receptors" in the muscle were unlikely to be nociceptive. Some unmyelinated fibres from the muscle were also found to be non-nociceptive (30). On the other hand, nociceptive afferents, when activated by severe muscle contraction, can also excite the SON neurons as well as cause respiratory and cardiovascular changes (31, 32).

The close relationship between excitation of neurosecretory neurons in the SON and plasma vasopressin level has been proved in several laboratories. Although the neurons studied in our experiment could not be definitely categorized as vasopressinergic because in cats, unlike in rats, vasopressinergic neurons have no distinct phasically firing pattern (33). On the other hand, the positive response to baroreceptor stimulation has been reported specific to vasopressinergic neurons (34), and because we found that 95% of neurons

excited by muscle nerve stimulation in our study were also inhibited by baroreceptor stimulation, the assumption that we recorded most often, if not exclusively, from vasopressinergic neurons seems valid. Thus, excitation of receptors in the muscle during contraction probably results in augmented vasopressin release through afferent action on hypothalamic neurons. It has already been suggested (6) that afferent muscle nerves must contribute to the increase in plasma vasopressin level seen during exercise because many changes, such as plasma osmolarity, plasma volume, and body temperative, etc., do not correlate well with changes of vasopressin level (3, 6, 7). In exercise afferent impulses originating from muscle receptors and acting directly on the SON could contribute, at least partly, to changes in vasopressin release.

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