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Responses of the Afferent Nerve from the Medial Gastrocnemius Muscle to the Intra-arterial Algesic Substances in the Cat†

Kwang Sup Sheen, Jun Kim* and Soon Dong Chung**

*Department of Physiology, Colleges of Medicine, Kyung Hee** University, Seoul 130-702 and Seoul National University, Seoul 110-460, Korea*

=Abstract= The afferent nerve fibers from the medial gastrocnemius muscle in the cat were studied and classified according to their conduction velocity and their responses to intra-arterial injection of KCl and lactic acid. Among a total of 513 units identified, 101 units were classified as group III and IV. Eighty-six units were tested for algesic substances. In 39 units there were no responses to either KCl or lactic acid. In 30 units which showed increased responses to KCl injections, responses to lactic acid were tested. Among them 11 showed an increase, four showed a decrease, and 15 showed no response. Among five units which showed a decreasing response to KCl, two showed an increased response to lactic acid, two a decreased response, and one showed no response. Eight units showed varying responses to KCl. Among them, four showed an increase, one showed a decrease, and one showed a decrease followed by an increased response to lactic acid. It was concluded from the above results that activation of the muscular afferent nerve fibers by intra-arterial injection of lactic acid, which could be produced during vigorous muscle contraction, might be related to muscle pain.

Key words: ***Muscle afferent nerve, Conduction velocity, Intra-arterial injection, KCl, Lactic acid***

INTRODUCTION

A number of chemical agents such as bradykinin, histamine, serotonin and potassium have been known to evoke pain in humans and pseudo-affective pain responses in experimental animals, and hence they were named as endogeneous algesic substances (Guzman *et al.*, 1962; Keele & Armstrong, 1964). These agents activate the nociceptors, and their information is carried by afferent nerve fibers of the Group III (Aδ, thin myelinated fiber) and Group IV (C, unmyelinated fiber) which are known to be involved in the transmission of nociceptive information (Mense & Schmidt, 1974; Franz & Mense, 1975). The majority of muscle afferent nerves belong to Group IV and Group III fibers (Boyd & Davey, 1968; Stacey, 1968; Boyd & Kalu, 1979). Hence most of the afferent nerves from the muscle seem to be related to muscle pain, although some of these fibers may carry non-nociceptive mechanical information.

Muscle pain can occur under various conditions which actually damage the muscle tissue or threaten to damage it. Probably some chemicals are released from the damaged cells to cause muscle pain. Among the chemicals that are released from tissues are such as potassium, hydrogen.

Potassium ions which are released from the intracellular space to the extracellular space during hypoxia, disturbances in the acid-base balance, and under inflammation and other tissue-damaging conditions have been known for

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*Author for correspondence

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many years to cause a sensation of pain (Moore *et al.*, 1933). The marked increase in afferent nerve fiber activity from the skeletal muscle when potassium ions were injected into that muscle (Mense, 1977) supports the above contention.

During muscle exercise under hypoxic or anaerobic conditions, a number of intermediate metabolites are produced and released from muscle cells (Lewis, 1932; Sahlin, 1986) to cause muscle fatigue. Particularly the accumulation of lactic acid lowers the muscle pH to 6.4-6.6. This acidic pH inhibits the excitation-contraction coupling as well as the activation process of muscle contraction and Na-K ATPase activity and causes muscle fatigue (Sahlin, 1986). Although administration of lactic acid in human subjects did not evoke an apparent cutaneous pain (Lindahl, 1961), lactic acid has a possibility of being one of the algesic substances adhering to the skeletal muscle where nerve endings are frequently exposed to the condition of lactic acid accumulation.

In the present study we attempted to observe changes in the muscle afferent nerve activity after an intra-arterial injection of lactic acid solution and compare them with those after injection of KCl and other algesic substances.

MATERIALS AND METHODS

Prepration of Experimental Animal

Twenty-one adult cats of both sexes (body weight: 2–3 kg) were used. Each cat was anesthetized with α-chloralose (60 mg/kg, i.p.), and canulas were inserted into the trachea, carotid artery and jugular vein. A single dose of 0.4 mg pancuronium bromide (Mioblock, from Organon) was administered through the venous canula for systemic muscle relaxation. The animals were then ventilated artificially with room air, and the end-tidal CO_2 pressure was monitored (Datex Normocap) and maintained within 4 $\pm$ 0.5% throughout the experiment. Arterial blood pressure was monitored with a physiological recording system (MX6 Recorder, from Devices). Body temperature was maintained within the range of 37 $\pm$ 1°C by using a heat controlled blanket (Animal Blanket Control Unit, from Harvard).

A laminectomy was done from the L4 to the S2 vertebrae, and the lumbosacral enlargement was exposed. The dorsal roots of L7 and S1 spinal segments were traced intradurally and cut near their entry zone to the spinal cord. After an incision was made in the back skin of the lower hind limb, nerves to the medial gastrocnemius muscle were exposed. Other lower limb nerves (femoral, obturator, hamstring, sural and common peroneal nerves) were cut so that the main sources of afferent inputs to the L7 and S1 dorsal roots would be from the medial gastrocnemius muscle. A catheter was inserted into the sural artery, and through this various algesic substances were injected, which were then flushed into the medial gastrocnemius muscle as well as other regions by arterial blood flow (Fig. 1). At the end of the experiment, a small dose of Evans blue was injected through the catheter to confirm the extent of distribution of the injected substances. When the surgical operation ended, the animal was then mounted on a stereotaxic frame, and a warm mineral oil pool was made around the exposed spinal cord and peripheral nerves.

Stimulation and Recording

A three-lead platinum hook electrode was placed in the medial gastrocnemius nerve and stimulated the nerve with square pulses of 0.5 ms, 0.5 Hz, 5 mA, which were sufficient to activate the Groups III and IV nerves. The L7 and S1 dorsal roots were cut and dissected under a surgical microscope until single-fiber activities could be recorded and mounted on a bipolar recording electrode. The signals were amplified (DAM 80 AC differential amplifier, WPI), monitored on an oscilloscope (VC-6041, Hitachi) and fed into a personal computer through a laboratory interface (CED 1401). When a single-unit activity was identified by conforming the action potential with constant latency to nerve stimulation, conduction velocity was determined and single-pass histograms were made using a computer program (CED 1401, M-rate neurological spike package).

Injection of Algesic Substances

All the substances were injected as a single dose of 0.15–0.3 ml of 1 M solution, and the remaining solutions in the catheter were flushed with 0.15 ml heparinized saline (30 units/ml) solution. The following substances were injected at random: KCl, NaCl, lactic acid, sodium lactate, ammonium chloride, a buffer solution (pH 3.3) and sodium bicarbonate.

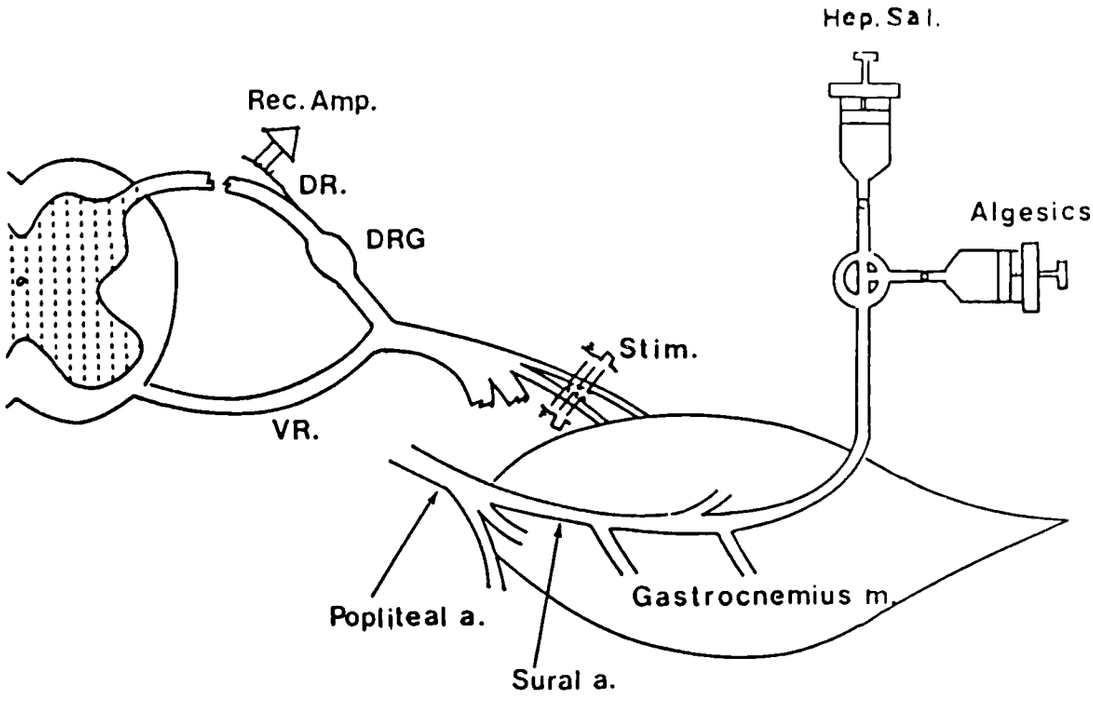

Fig. 1. Schematic diagram of experimental set-up. The hind limb below the hip was denervated except for the nerves to medial gastrocnemius muscle which were left intact and mounted on platinum tripolar stimulating electrode. Sural artery was cannulated to inject various algesic substances. L7 and/or S1 dorsal rootlets were cut centrally and dissected into fine fascicles. Dissected dorsal root fibers were placed on a platinum bipolar recording electrode. Recorded electrical activities were amplified and displayed on an oscilloscope. DR: dorsal root, DRG: dorsal root ganglion, VR: ventral root.

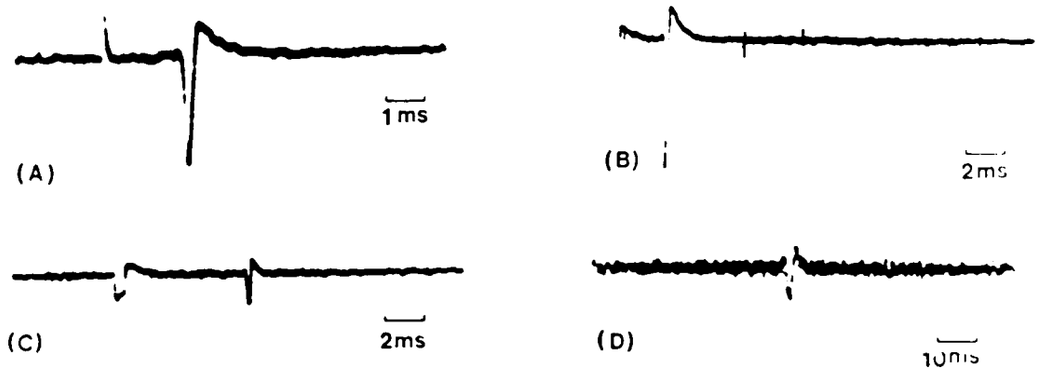

Fig. 2. Examples of single-unit activities recorded in dorsal root fascicles while stimulating medial gastrocnemius nerves (0.5 Hz, 0.5 msec, 5 mA). Conduction velocities of the fibers in panels A, B, C and D were 88.9, 58.3, 22.0 and 2.5 m /sec in the order which represents Group I, II, III and IV fibers, respectively.

RESULTS

The first step of the experiment was the isolation of single unit activities and group of them. Fig. 2 shows some examples of the single-unit activities evoked by the electrical stimulation of the MGS nerves (0.5 ms, 5 mA, 0.5 Hz). The conduction velocities of the shown units were 88.9, 58.3, 21.7 and 2.8 m/sec, respectively, in a panels A, B, C and D, in that order. Hence each unit belonged to Groups I, II, III and IV. In Fig. 3, a total of 359 single-fiber units activated by the electrical stimulation of the MGS nerves and recorded in the cut L7 and S1 dorsal root fascicles in five cats were compiled to make a histogram of conduction velocity. Among them,

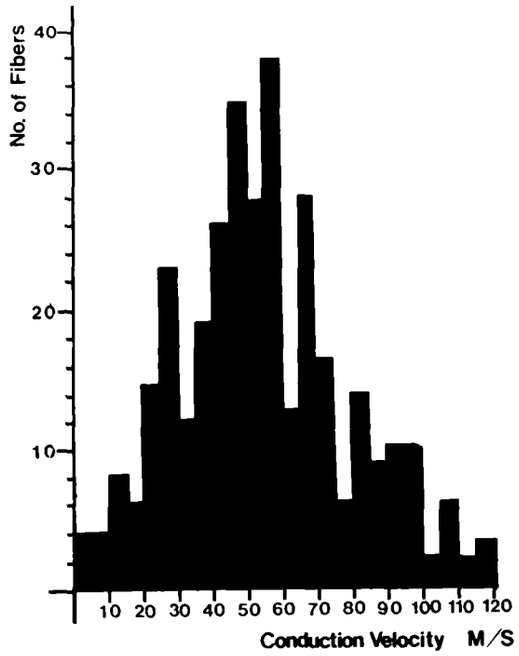

Fig. 3. A histogram of conduction velocity compiled from 359 single afferent fibers from medial gastrocnemius muscles in five cats. The abscissa and ordinate represent the conduction velocity and the number of fibers.

88 units belonged to Group I(24.5%), 215 units to Group II(59.9%) and the remaining 56 units to Groups III and IV, which are thought to carry nociceptive information.

When a single-unit activity was successfully identified, various algesic substances were administered through the sural arterial catheter to activate the recorded unit chemically. Fig. 4 represents an example of such an experiment. The peristimulus time histogram was made by counting the number of impulses for 600 seconds with 1 second interval and the figure shows the responses of an muscle afferent fiber (Group III, conduction velocity: 30 m/sec) to intra-arterial injection of KCl and lactic acid. The resting discharge of the unit, observed during the first 50 seconds period was 4.9 impulses/sec. The unit showed clear and notable responses to 0.3 ml of 1 M KCl given at 50 sec from the beginning and 1 M lactic acid at 300 msec, with less than a 10-sec delay. Peak dischage rates for KCl and lactic acid were 110 and 42/sec. After that the discharge rate decreased even below the control level in about 200 seconds.

Not all the recorded units, however, showed such typical responses of excitation to both KCl and lactic acid. A total of 82 units were tested for both KCl and lactic acid. In 39 units there

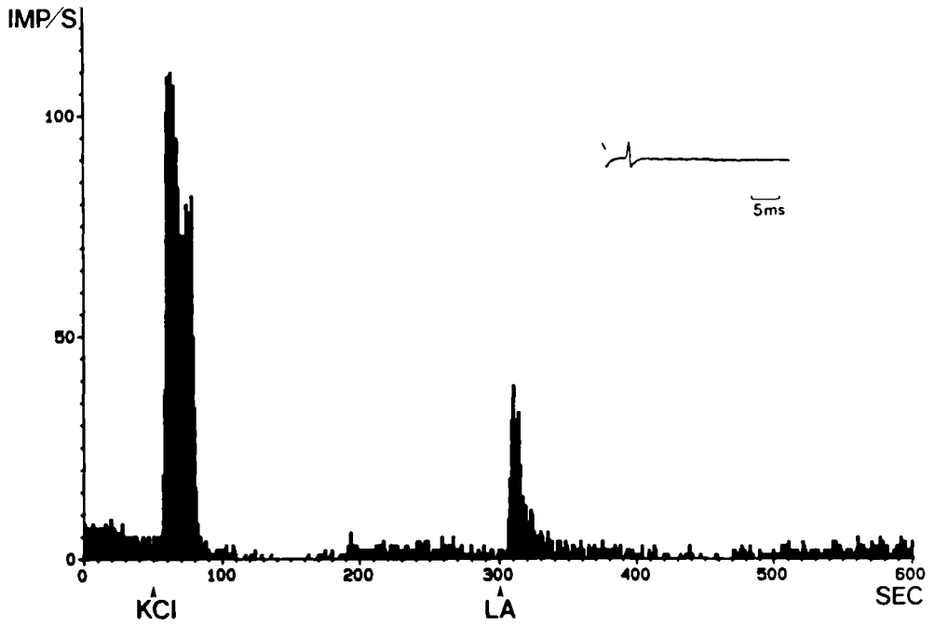

Fig. 4. A single pass time histogram obtained from a single afferent fiber responding to intra-arterial injection of KCl and lactic acid. After a resting period of 50 sec., a 0.3 ml solution of 1M KCl was injected and a lactic acid solution was injected at 300 sec., from the beginning. Conduction velocity of this unit was 30 m/sec. Inset shows an action potential of this unit. Arrows indicate the time of single-shot injections which were completed in about 5 sec. Ordinate: number of impulses/bin (bin width, 1 sec); abscissa: time in seconds.

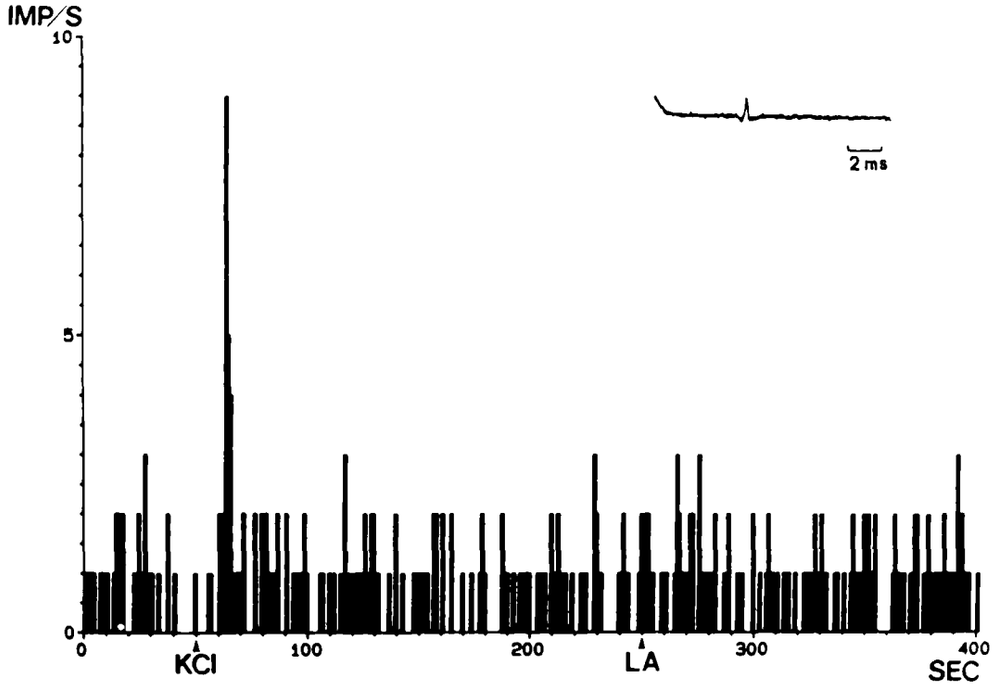

Fig. 5. A histogram of a single Grop III fiber (conduction velocity, 18.7 m/sec) responding only to intra-arterial KCl but not to lactic acid.

was no response to either the KCl or lactic acid injections. Thirty-eight units elicited an increased response to 1 M KCl. Only in 15 units did the impulse discharge increase in response to the lactic acid injection. In six units intra-arterial injection of 1 M KCl increased the discharge rate, while the 1 M lactic acid solution decreased the rate as shown in Fig. 5. In 17 units which showed an increased response to 1 M KCl, there was no response to the lactic acid solution.

In five units among 43 units which showed any response to 1 M KCl, intra-arterial injection of the 1 M KCl solution depressed the resting activity. These units weren't the deteriorating ones since the injection of lactic acid after 1 M KCl exerts apparent effects. Fig. 6 represents one example of such a unit. Before the 1 M KCl injection, the resting discharge rate was 18.7/sec. At 50 sec when KCl was injected, the impulse discharge slowed down to 5.5/sec in 200 sec. Lactic acid, which was injected at 370 sec, increased the rate markedly to 165/sec within 50 sec. The effect was sustained for about 100 sec.

To observe the mechanism of the effect of lactic acid on the single muscle afferent fiber activity, solutions of 1 M KCl, lactic acid and other chemicals, whose effects could be compared with that of lactic acid, were injected through the sural artery. In Fig. 7, a Group II fiber with a conduction velocity of 58.3 m/sec was studied. The resting discharge rate was 53.4/sec, and 0.3 ml of 1 M KCl solution injected at 50 sec increased the rate beyond 300/sec in less than 20 sec. It then decreased the rate to swing below the resting level and then slowly recovered in 90 sec after a KCl injection. At 250 sec, 0.3 ml of lactic acid was injected, and the discharge rate went up to 130/sec in 5 sec but soon came down to 35/sec. When a sodium lactate solution was injected at 350 and 700 sec, the discharge rate increased by 20 impulses/sec with a latency of 20 to 30 sec. Injection of ammonium chloride at 550 sec also increased the discharge rate with a latency of about 50 sec. Fig. 8 shows another example of such an experiment. The resting discharge rate of this unit was 2.4/sec. An injection of 1 M KCl solution increased the rate up to 145/sec in 20 sec and was maintained for about 120 sec. Lactic acid also increased the rate, but solutions of buffer (pH = 3.3) at 400 sec, sodium lactate at 550 sec, NaCl at 720 sec and NH_4Cl at 850 sec

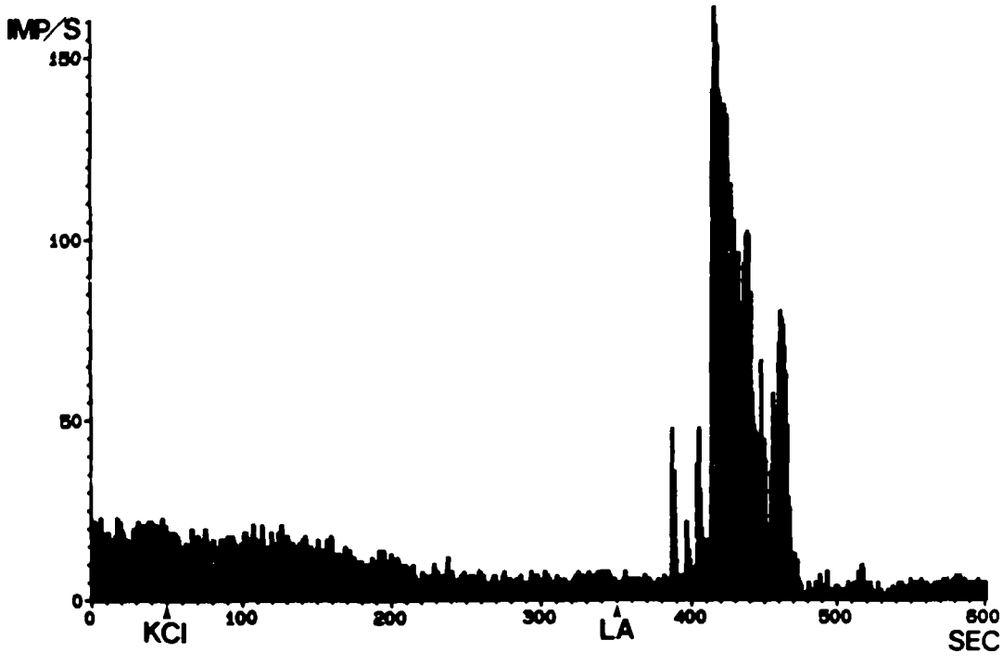

Fig. 6. A histogram of a single unit responding to intra-arterial KCl and lactic acid. To KCl it showed slow decreasing tendency of discharge, but to lactic acid the discharging rate went up rapidly. KCl was injected at 50 sec and lactic acid at 250 sec.

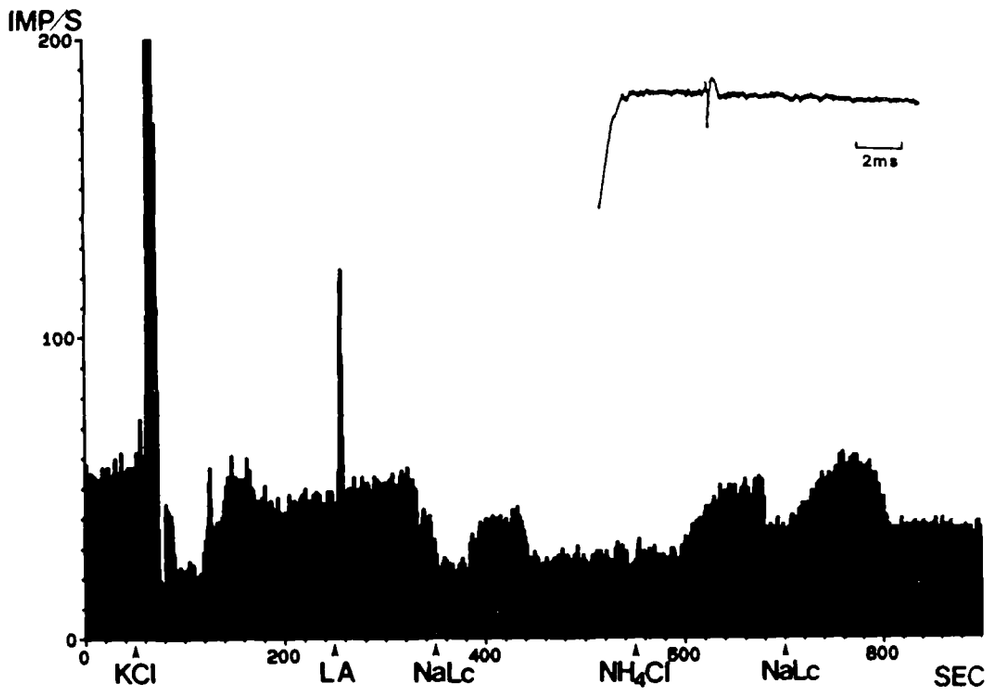

Fig. 7. Comparison of the effect of lactic acid on a single afferent unit from medical gastrocnemius muscle with those of KCl, sodium lactate and NH_4Cl. Arrows indicate the time of injection.

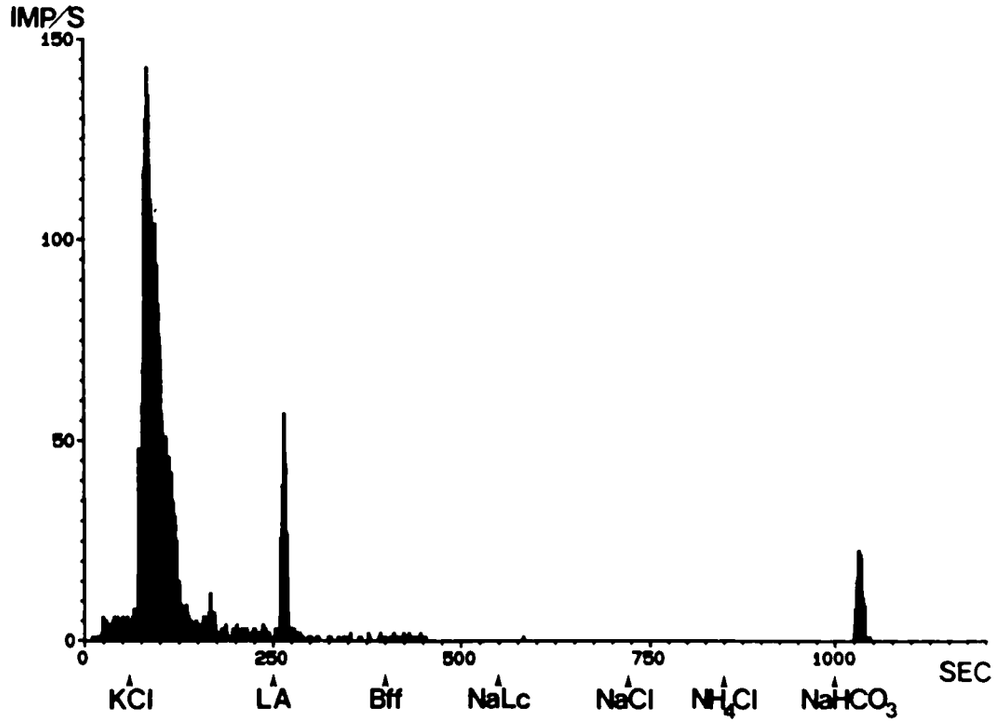

Fig. 8. Comparison of the effect of lactic acid on a single afferent unit with those of KCl, a buffer solution of pH 3.3, sodium lactate, NaCl, NH_4Cl and sodium bicarbonate.

did not elicit any change, and the unit did not discharge at all. This unit was not dead, however, since it responded to $NaHCO_3$ solutions injected at 1000 sec and discharged up to 25 impulses/sec. These results might suggest that the effect of sodium lactate on the muscle afferent nerve is mediated by changes in the intracellular pH.

DISCUSSION

According to the diameter and conduction velocity of the impulses, peripheral nerves are classified into groups: Group I with a diameter of 12-20 μm and a conduction velocity of 72-120 m/sec; Group II with 2-16 μm and 30-72 m/sec; Group III with 1-6 μm and 4-30 m/sec and, finally, Group IV with less than 1 μm and slower than 2.5/sec (Willis & Grossman, 1981).

Although all of the above group nerve fibers were identified in mammalian skeletal muscles, Stacey (1968), in his morphological study, reported that cat skeletal muscle afferent fibers belonging to group IV outnumbered the sum of the remaining fibers of Group I-III. According to a more detailed study (Boyd & Davey, 1968), in cat medial gastrocnemius musle the ratio of myelinated (Groups I-III) fibers to unmyelinated ones (Group IV) was 3:7, and the ratios of afferent to efferent fibers were 60:40 in myelinated fibers and 57:43 in unmyelinated fibers. For afferent nerves, the ratio of Group I and II fibers to Group II and IV fibers was 20.8:79.2.

Our electrophysiological study revealed that Groups I and II fibers were 84.4 % and Groups III and IV fibers were 15.6 %. We think, however, that this discrepancy is not a true discrepancy, since the electrophysiological method has an innate bias for easier recording of larger-diameter fibers than smaller ones.

Extreme exercise or some pathological conditions (e.g., inflammation and necrosis) frequently result in a sensation of pain. Under these conditions, the osmolarity and hydrogen ion concentrations are usually elevated. In inflammatory tissues causing pain, a number of algesic substances were identified, for example, bradykinin, kallikrein, serotonin and histamine. Injection of these agents, however, is not sufficient to cause pain since simultaneous inhibition of release and /or uptake of potassium with injection of these agents results in little pain. A Study made by Foreman *et al.* (1978), which revealed that mus-

cle afferent nerve activities increased much more during injection of KCl than that of bradykinin into the muscle, further supports the above contention. Variations in osmolarity and hydrogen ion concentrations in muscle tissue can also result in pain (Lindahl, 1961).

In the present study when KCl and lactic acid were injected into the artery to the medial gastrocnemius muscle, the response of the muscle afferent nerve fibers varied. Some units did not response at all to both agents, some responded only to KCl or to lactic acid, and others responded to both. Both increasing and decreasing responses could be seen in the responding units, and in some cases the units showed a complex response of an increasing and then decreasing discharge rate and vice versa. These results suggest that muscular afferent nerve fibers belonging to the same nerve fiber group do not mean that they are functionally homogenous ones, and a single group of nerve fibers might have several smaller functional subpopulations. This contention is further supported by studies that show: (a) Among Groups III and IV fibers, which were thought to carry nociceptive afferent inputs, only two-thirds of the Group III and one half of the Group IV nerves were responsive to algesic substances (Mense, 1977); (b) One single unit responded variously to several algesic substances (Mense & Schmidt, 1974); (c) Slow-conducting afferent units in cat skeletal muscles were grouped into at least four types according to their responses to mechanical and thermal stimulation (Mense & Meyer, 1985). In addition to nociceptive transmission, these groups of fibers play a role in cardiopulmonary adjustments occurring during muscle exercise (Johansson, 1962; Coote & Perez-Gonzales, 1971, McCloskey & Mitchell, 1972).

It is now accepted that the accumulation of metabolites in the muscle is one of the factors responsible for muscle pain (Mills *et al.*, 1984). Although patients with myophosphorylase deficiency still report pain during ischemic exercise (Schmid & Hammaker, 1961), lactic acid has been thought by many to be one of the algesic substances. In the present study, one-half and one-fourth of the units studied by intra-arterial injection responded to KCl and lactic acid, respectively. The pH of the lactic acid solution used was 3.3, but as shown in Fig. 8, the buffer solution of pH 3.3 was not effective in evoking afferent activity. Hence the response to lactic acid did not seem to be mediated by changes in extracellular pH. When the effect of lactic acid was compared to those of sodium lactate, NH_4Cl, $NaHCO_3$ and NaCl, only the sodium lactate resulted in a response similar to lactic acid. NH_4Cl sometimes resulted in activation of muscle afferent activity as shown in Fig. 7, but the duration was much slower than that of sodium lactate. Since an intra-arterial injection of sodium lactate solution could result in intracellular acidosis (Sharp & Thomas, 1981; Mason & Thomas, 1988), and NH_4Cl results in intracellular alkalosis and removal of it results in acidosis (Boron, 1977), the results of the present study suggest a possibility that the mechanism of muscle afferent activation by intra-arterial lactic acid and NH_4Cl injection involves an intracellular acidification of the receptors. This mechanism is analogous to the role of lactic acid accumulation in muscle fatigue (Hermansen & Osenes, 1972).

If we compare the effect of lactic acid with KCl in units which showed response to both agents, the latencies were 17.2 sec in KCl and 23.2 sec in lactic acid. Peak times were 39.2, 80.4 sec, and the discharge rates were 44 and 15 times more than those of the control, respectively. Considering the fact that KCl might act directly on the membrane to depolarize it, the slower duration of the lactic acid effect might be due to intracellular processes.

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= 국문초록 =

고양이에서 통각물질의 동맥내 주입후 유발되는 내측비복근 감각신경의 활동

서울대학교 및 경희대학교* 의과대학 생리학교실

신광섭 · 김 전 · 정순동*

고양이의 내측비복근 구심신경을 흥분전도속도에 따라 종류를 결정하고 동맥을 통해 KCl과 락트산 등 유해자극물질을 투여하였을 때 유발되는 구심신경 impulse의 변화를 관찰하였다. 총 513개의 확인된 구심섬유 중에서 제 III, IV군에 속하는 것은 101개 이었다. 이중 86개에서 동맥내로 통각물질을 투여하였는데 그중 39례는 이렇다할 변화를 보이지 않았다. 30예에서는 KCl투여로 흥분이 증가하였는데 이중 11예는 락트산으로도 증가하였고 4예는 감소 15예에서는 락트산에 이렇다할 변화를 보이지 않았다. 5예에서는 KCl투여로 흥분발사가 감소하였는데 이중 2예는 락트산에 의해 증가하였고 2예는 감소, 1예는 별다른 변화가 없었다. 8예는 KCl에 대하여 증가 후 감소하였는 바 이중 4예는 락트산에서 증가, 1예에서 감소, 1예는 감소 후 증가 그리고 2예는 변화가 없었다. 이상의 결과는 통각을 전달한고 알려진 제 III, IV군의 신경들이 통각물질들에 대해 동일한 반응을 보이는 않으며 락트산에 반응을 보이는 제 III, IV군의 신경들이 많은 것으로 보아 근육의 과도한 수축시에 발생하는 락트산에 의하여 활성화되는 구심신경의 활동이 근육의 통증과 관련이 있을 것으로 사료된다.