Cholinergic Mechanism in the Inhibition of Spinal Reflexes from the Superficial Radial Nerve of the Decerebrate Cat

It has been known that stimulation of the superficial radial (SR) nerve depresses some activity of the central nervous system, for example, low rate (3–8 Hz) volley produces EEG synchronization[1], and depresses or abolishes extensor and flexor EMG responses to stimulation of proprioceptive muscle afferents[2], and further, a single volley evokes the inhibitory postsynaptic potentials (IPSPs) not only in the trigeminal but also in extensor and flexor lumbar spinal motoneurones[3]. Recently we found that the IPSPs in the trigeminal motoneurones were increased in size and late hyperpolarization newly appeared after an administration of physostigmine (0.15 mg/kg, i.v.) and that these physostigmine effects were antagonized by atropine (1.0 mg/kg, i.v.) but not by dihydro-β-erythroidine (0.5 mg/kg, i.v.)[4]. In the present study we attempted to investigate the effects of physostigmine and related compounds upon the inhibition of the spinal reflexes from the SR nerve in unanesthetized decerebrate cats.

Materials and methods. All surgical operations were performed under ether anesthesia. Cats were decerebrated precollicularly by suction and the spinal cord was exposed from L_5 to S_2. The ventral roots of L_5–S_1 were cut and the spinal reflexes were recorded from L_5 and L_7 ventral roots. Polyethylene catheters were inserted to the contralateral femoral artery (for monitoring the blood pressure) and femoral vein (for drug administration). After the operations were completed, ether anesthesia was discontinued and the cats were immobilized with gallamine triethiodide and were maintained under artificial respiration (30–40/min). Experiments were started at least 3–4 h after the discontinuation of the anesthesia. Both rectal and spinal pool temperatures were kept at 37–39°C with an automatic thermoregulator deviced in our laboratory.

Results and discussion. A single shock stimulus to either one of the tibial, peroneal and medial gastrocnemius-soleus (mG-S) nerves induced monosynaptic reflex (MSR) in L_7 and polysynaptic reflex (PSR) with the latency of about 4 msec in L_5 ventral roots (Figure 1 H). As reported previously[3] and exemplified in Figure 1A, B and F (upper trace), both MSR and PSR were depressed after a phase of transient enhancement by the conditioning stimulation of either ipsilateral (A) or contralateral (B) SR nerve at a supramaximal intensity for Aα afferent fibers. The initial enhancement of the MSR and PSR (cutaneous facilitation) appeared at a latency of about 15 msec, attained maximum at about 25 msec and declined gradually by about 35 msec. Immediately after the cutaneous facilitation, the inhibition of the MSR and PSR were observed (cutaneous inhibition), reached maximum at about 50 msec and decayed with time in 85–90 msec. It is rather rare to obtain the cutaneous inhibition, however, as is illustrated in Figure 1 E and F, it was potentiated by an administration of physostigmine (0.15 mg/kg) regardless of the size of the existing inhibition before physostigmine (D, G). This physostigmine effect appeared in 1–2 min after administration, attained maximum at 4–15 min and gradually declined in about 30–40 min.

Another effect of physostigmine at the same dose in decerebrate cats was the diminution of the MSR and PSR in size with a similar time course to its potentiating effect on the cutaneous inhibition. The maximum reduction of the MSR in amplitude was about 30–40% of the control. In low spinal cats, however, the same dose of physostigmine produced little change in either MSR or PSR. Therefore, it is considered that the depression of the MSR and PSR may be due to the descending influences from supraspinal structures. Both of these physostigmine effects were completely blocked by atropine (1.0 mg/kg) (E) but not by dihydro-β-erythroidine (0.5 mg/kg). This may indicate that muscarinic, rather than nicotinic, cholinergic mechanism is concerned with the cutaneous inhibition.

In order to clarify which part of the brain stem was concerned with the cutaneous inhibition, either medial or

1 O. POMPEIANO, and J.E. SWETT, Experientia *17*, 323 (1961).
2 S. GIAQUINTO and O. POMPEIANO, Arch. ital. Biol. *102*, 393 (1964).
3 K. KUBOTA, Y. KIDOKORO and J. SUZUKI, Jap. J. Physiol. *18*, 198 (1968).
4 N. IWATA, Y. SAKAI and T. DEGUCHI, Expl. Brain Res., in press (1971).

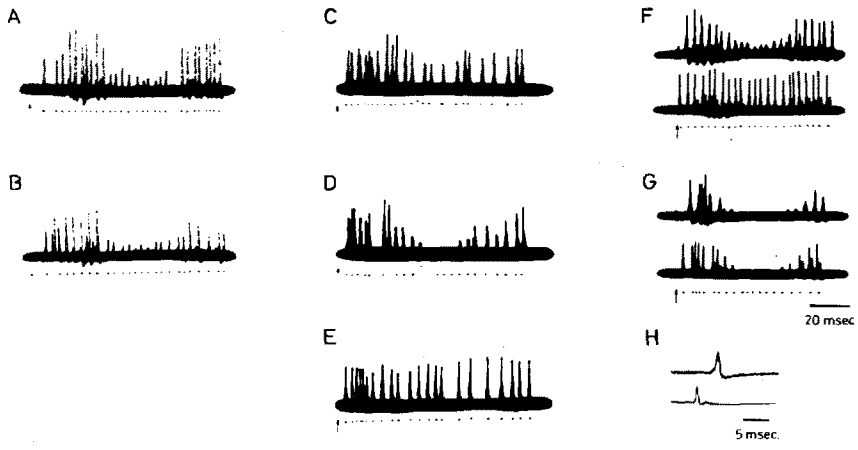

Fig. 1. Time course of the cutaneous inhibition and effects of physostigmine. A to B, C to E and F toH were obtained from different animals, respectively. A, B: typical example of the cutaneous facilitation and inhibition of the MSR produced by the conditioning stimulation of the ipsilateral SR nerve (A) and contralateral one (B). C to E and lower traces of F to H: the cutaneous inhibition of the MSR recorded from L_7. Upper traces of F to H: that of the PSR recorded from L_5 ventral root. These reflexes were evoked by stimulation of the tibial nerve. C, F: before. D, G: 5–10 min after physostigmine (0.15 mg/kg). E: 3 min after atropine (1.0 mg/kg). H: MSR (lower trace) and PSR (upper trace) at fast sweep speed. Upward arrows: time of stimulation of the SR nerve. Dots in A to G: that of the tibial nerve.

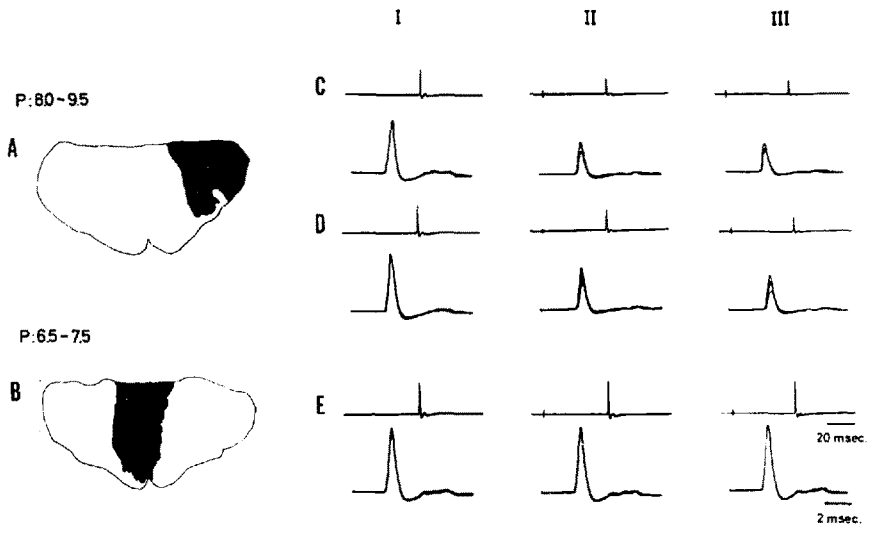

Fig. 2. Effect of brain stem lesion upon the cutaneous inhibition. A and B; filled areas are part of lesion of brain stem at indicated position. Column I; MSR evoked by the tibial nerve stimulation alone. Column II; MSR after the conditioning stimulation of the ipsilateral SR nerve at an interval of about 50 msec. Column III; MSR after that of the contralateral SR nerve at the same interval as Column II. Line C, before; Line D, after the lesion of lateral part of the medulla shown in A. Line E; after lesion of medial part shown in B. Upper traces were obtained at a slow sweep speed and lower ones at a fast sweep speed.

lateral part of the brain stem was cut with fine scissors after the removal of the cerebellum by suction under physostigmine effective state. As seen in Figure 2, the MSR had been depressed to about 50% by the conditioning stimulation of either ipsilateral or contralateral SR nerve at a conditioning-testing interval of about 50 msec before lesion in this preparation (C). After lesion of the lateral part of the medulla (A) the cutaneous inhibition changed little (D), while it was completely abolished by section of the medial part of it (B, E). This clearly shows that the medial part of the medulla is concerned with the cutaneous inhibition, but the localization of physostigmine and/or acetylcholine sensitive mechanism remains unknown.

Zusammenfassung. Nachweis einer verstärkten Spinalreflex-Hemmung bei der decerebrierten Katze durch Physostigmin. Der Reflex wird über N. radialis superficialis hervorgerufen und durch Atropin völlig aufgehoben, während Dihydro-β-erythroidin keine Wirkung hatte.

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Effect in vivo of Norepinephrine on the Membrane Resistance of Brown Fat Cells[1]

The cold-induced increase in thermogenesis of brown fat appears to be mediated by norepinephrine (NE) derived from sympathetic nerve terminals[2]. One of the first events measurable in this metabolic activation is the depolarization of the membrane of the brown fat cell. This change in membrane potential can be elicited by NE applied either in situ[3] or in vitro[4,5]. Similar changes are also seen following electrical stimulation (in vivo) of the sympathetic nerves to the brown fat pad[3]. That this depolarization is at least partially associated with activation of adenyl cyclase was suggested[3] following the finding that: a) the depolarization induced by electrical stimulation is abolished after treatment of the animal with the adrenergic antagonist, propranolol; and b) theophylline, although eliciting a thermogenic response from the brown fat, did not result in a depolarization of the membrane[3]. Recently, observations obtained in vitro have confirmed these results and have been similarly interpreted[5].

As an explanation of the underlying basis for this depolarization, 2 possible mechanisms may be considered; namely, an increase in membrane permeability and/or a direct change in the activity of an electrogenic pump (Figure 1). Hence, the present study was undertaken to determine whether the NE-induced shift in ionic distribution was indeed accompanied by an increase in the permeability of the membrane.

To evaluate this possibility, the effect of NE on the membrane resistance of the brown fat cells was examined in Long-Evans, cold-acclimated (4 ± 1°C, 3–4 weeks) rats. These rats were anesthetized (sodium pentobarbital,

[1] Supported in part by research grant NASA No. NGR-05-004-035.
[2] R. E. Smith and B. A. Horwitz, Physiol. Rev. *49*, 330 (1969).
[3] B. A. Horwitz, J. M. Horowitz jr. and R. E. Smith, Proc. natn. Acad. Sci., USA *64*, 113 (1969).
[4] L. Girardier, J. Seydoux and T. Clausen, J. gen. Physiol. *52*, 925 (1968).
[5] G. Krishna, J. Moskowitz, P. Dempsey and B. B. Brodie, Life Sci., *9* (Part I), 1353 (1970).