impermeability of methylhydroxycoumarin through the blood brain barrier was due to the predominance of impermeable metabolite, sulfate conjugated form, in plasma. Furthermore, it should be noted that the brain level of free methylhydroxycoumarin decreased parallel with the decrease in the plasma level, even though the plasma level was higher than that in the brain. This indicates either one of the following two cases: 1. the presence of a mechanism which extrudes methylhydroxycoumarin from the brain. If this were the case, penetration of free methylhydroxycoumarin into the brain should be better than the observed value, or, 2. in some compartment of the brain, which is accessible only by the free form, the level of free methylhydroxycoumarin is very close to that in the plasma and a rapid equilibrium exists between this compartment and the plasma.

In either case, free methylhydroxycoumarin, which is rather low in liposolubility, penetrates through the blood brain barrier easily, although its lipid insoluble metabolite, the conjugated form, does not gain access to the brain. Thus, the threshold of the blood brain barrier, in terms of liposolubility, was lower than that generally believed. It may be that the limited access to the brain of more liposoluble substances is due to the faster metabolism of those compounds into polar forms. It may be postulated that the accessibility of drugs to drug metabolizing enzymes in the liver increases with the increasing liposolubility (7).

REFERENCES


ACTIONS OF CERTAIN POLYPEPTIDES
ON FROG SPINAL NEURONS

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Pharmacological actions of polypeptides have recently been extensively studied. However, relatively little is known about the effects of polypeptides on neurons of central nervous system (CNS). Several pharmacologically active polypeptides are known to exist in mammalian CNS (1). Furthermore, Lembeck and Zetler suggested the possible transmitter role of Substance P which is known to be polypeptide(s) in nature (2). In the present
study, we, therefore, investigated the actions of certain polypeptides on frog spinal neurons.

Isolated and hemisected spinal cord of bull frog (*Rana catesbiana*) was placed in a bath containing 0.3 ml Ringer solution which was oxygenated and kept at 15±1°C. 8th or 9th dorsal root was electrically stimulated at a rate of 0.1 c/s, and potential changes generated in spinal motoneurons were recorded as described by Curtis et al. (3). The main bath containing the spinal cord was grounded through a calomel electrode. 8th or 9th ventral root ipsilateral to the stimulated dorsal root was drawn through a small hole in the lateral wall of the main bath into a paraffine trough and placed on Ag-AgCl electrode, which was connected through a DC amplifier to an oscilloscope and a pen-recorder.

Physalaemin exerted a strong depolarizing action on spinal motoneurons. In Fig. 1, the potency of physalaemin action to induce a depolarizing potential change was compared with that of L-glutamate. The excitatory action of physalaemin was about 500 times stronger than L-glutamate on a molar basis. When the synaptic transmission in the spinal cord was blocked by tetrodotoxin (10⁻⁷ g/ml) or by lowering calcium concentration in the medium (0.2 mM), the depolarizing action of physalaemin was unchanged or rather potentiated. The depolarizing action of L-glutamate, on the other hand, was slightly reduced by tetrodotoxin and potentiated in 0.2 mM-Ca medium. These results suggest that physalaemin as well as L-glutamate has a direct depolarizing action on spinal motoneurons.

Bradykinin also produced a depolarizing action on spinal motoneurons. This action of bradykinin was about 10 times stronger than L-glutamate, and was almost completely blocked by tetrodotoxin (10⁻⁷ g/ml) or by reducing calcium concentration (0.2 mM). Therefore, bradykinin seems to act on spinal motoneurons trans-synaptically, probably by excit-
ing the interneurons.

Angiotensin I and II showed a similar depolarizing action with pronounced tachyphylaxis. Details of their actions were not studied. When physalaemin and bradykinin were incubated with chymotrypsin, their actions on the spinal neurons were completely abolished.

The present results show that certain polypeptides exert strong excitatory effects on frog central neurons, and further suggest that different polypeptides may act on different types of neurons. The excitatory action of physalaemin is particularly strong, several times stronger than the action of N-methyl-D-aspartate which is the strongest among the known excitatory substances on frog spinal cord (3). Physalaemin is known to exist in the skin of certain frogs, and to have a strong vasodilator action (4). It has been suggested that the chemical structure of physalaemin may be closely related to that of Substance P (5). These informations altogether suggest that physalaemin may possibly be related to the excitatory transmitter in the spinal cord.

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