# SENSITIZATION OF DORSAL ROOT FIBERS BY BRADYKININ AND ACETYLCHOLINE TO STIMULATION BY POTASSIUM IONS

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Acetylcholine (AC) and bradykinin (BR), in sufficiently high concentrations, induce excitation of chemosensitive dorsal root fibers, as shown by the appearance of pressor reflexes. In low concentrations these substances induce subthreshold depolarization, revealed by an increase in the sensitivity of the chemisensitive dorsal root fibers to stimulation by potassium ions. Threshold AC concentrations for the sensitizing effect are 0.01-0.1 μg/ml, and the corresponding concentrations of BR are 0.001-0.01 μg/ml, i.e., they are indistinguishable from threshold doses for excitation of the tissue receptors. In the case of AC these concentrations are 500-1,000 times, and of BR 1,000-5,000 times lower than threshold concentrations for direct excitation of afferent fibers. After treatment with BR, the threshold KCl concentration for excitation of afferent fibers is reduced to the characteristic values for the potassium ion concentration in foci of inflammation. Since in such foci kinin formation is increased, this suggests that the effect of sensitization of the fibers to the action of potassium ions by BR may play a role in the genesis of inflammatory hyperalgesia.

Among the dorsal root fibers there is a group of chemosensitive fibers [10, 15] whose properties have as yet received little study. By contrast with the peripheral nerves, the connective-tissue sheaths of the dorsal roots are poorly developed. The chemosensitive properties of the dorsal root fibers can therefore be investigated by application of the stimulus to their surface. A bundle of these fibers can be regarded as a convenient model for the study of certain properties of the chemosensitive receptor zones of the body.

It has previously [5] been shown that dorsal root fibers are excited by potassium ions in concentrations of about 20 mM and above. Acetylcholine and bradykinin are known to increase the sensitivity of peripheral afferent nerves to stimulation by potassium ions [2, 4].

The object of this investigation was to determine whether the sensitivity of dorsal root fibers to stimulation by potassium ions can be increased by the action of acetylcholine (AC) and of bradykinin (BR).

## EXPERIMENTAL METHOD

The pressure in the carotid artery was recorded in cats anesthetized with urethane (20% solution). Solutions of the stimuli were applied to dorsal root L7 by the method described previously [5]. In each experiment the action of potassium ions were studied in subthreshold, threshold, and superthreshold concentrations before and after application of AC and BR to the roots. The action of AC was tested in eight experiments and that of BR in nine experiments. During each successive experiment the concentration of these substances was increased by 5-10 times. In the first three experiments of each series the presence of a sensitizing effect was established (for AC in concentrations of between 10 μg and 10 mg/ml, for BR in

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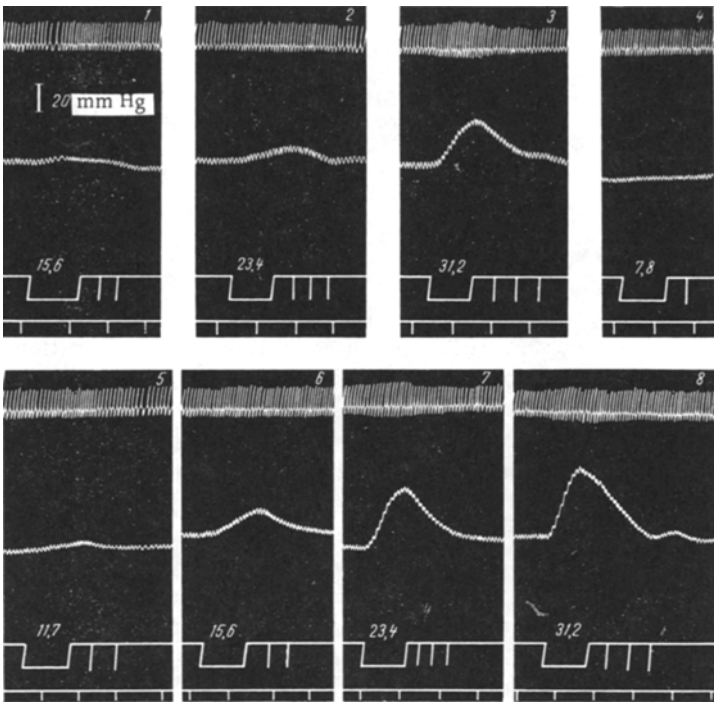


Fig. 1. Pressor reflexes arising in response to the action of potassium chloride on the dorsal root and after application of AC to it in a concentration of 50 μg/ml. From top to bottom: respiration, arterial pressure, marker of stimulation, time marker (30 sec). Zero line coincides with time marker. Numbers above marker of stimulation show concentration of potassium chloride (in mM).

concentrations of between 0.1 and 10 μg/ml), while in the next experiments the threshold concentrations for this effect were determined (for AC, starting with a concentration of 0.01 μg/ml, and for BR starting with a concentration of 0.001 μg/ml).

## EXPERIMENTAL RESULTS

After application of AC to the dorsal root pressor reflexes appeared in six animals. The threshold concentration for this effect was 10-50 μg/ml. With an increase in concentration the reflexes were strengthened, to reach a maximum at 1 mg/ml. In two experiments no reflexes appeared even in response to a concentration of 10 mg/ml. Bradykinin induced pressor reflexes in only three of nine experiments, and then only in relatively high concentrations (5-10 mg/ml). The increase in intensity of the pressor reflexes induced by bradykinin was characteristically slow. The results obtained by the action of KCl on the dorsal roots were the same as those obtained previously: the mean threshold concentration was about 20 mM [5].

The sensitizing action of AC on the dorsal root fibers is illustrated in Fig. 1. The threshold concentration of potassium ions for the dorsal root was 15.6 mM. After the action of AC in a concentration of 50 μg/ml the threshold concentration of potassium ions was lowered to 11.2 mM. The pressor reflexes to superthreshold concentrations of KCl also were intensified. Threshold concentrations of AC for the sensitizing effect were 0.01-0.1 mg/ml, i.e., 500-1,000 times below those for an excitatory effect. The sensitizing effect of AC also was exhibited in the two experiments in which it did not induce pressor reflexes.

An increase in the sensitivity of the dorsal root fibers to stimulation by KCl was produced by BR in all nine experiments (Fig. 2). Threshold concentrations of BR for this effect were 0.001-0.01 μg/ml, which is 1,000-5,000 times below the threshold for the excitatory effect. In response to the action of BR

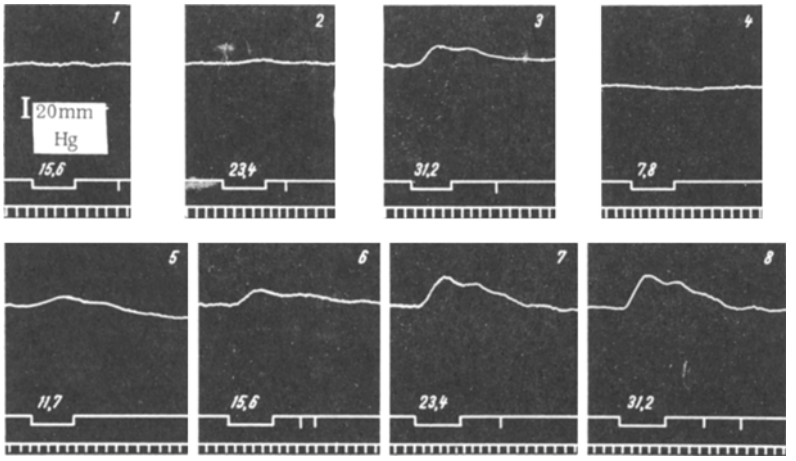


Fig. 2. Pressor reflexes arising in response to the action of potassium chloride on the dorsal root before and after application of BR to it in a concentration of 1 mg/ml. From top to bottom: arterial pressure, marker of stimulation, time marker (5 sec). Remainder of legend as in Fig. 1.

in concentrations of 0.5 μg/ml and above, just as in experiments on the mesenteric nerves [4], reflexes appeared to subthreshold KCl concentrations: in this case to 11.7 and 15.6 mM.

Sensitization increased with an increase in the BR concentration. In some experiments, however, after application of BR in a concentration of 10 μg/ml the reflexes in response to potassium ions were increased by a relatively smaller amount. This decrease in the effectiveness of the sensitizing action of BR was evidently due to the tachyphylaxis which is a characteristic feature of this substance: a decrease in the effects induced by it on repetition of its action [3, 4]. It must be emphasized that the increase in the sensitivity of the dorsal root fibers to potassium ions induced by BR is very considerable: the threshold concentration of KCl was reduced by 33-50% compared with that before the action of BR, while the superthreshold reflexes were strengthened by 6-7 times. The increased sensitivity of the dorsal root fibers to potassium ions after treatment with AC or BR persisted for several minutes.

## DISCUSSION

In low concentrations AC and BR thus increase the sensitivity of dorsal root fibers to the excitatory action of potassium ions. It is highly significant that concentrations which are threshold for the sensitization effect are indistinguishable from threshold concentrations for excitation of the tissue receptors: 0.01-0.1 μg/ml for AC [1, 2] and 0.001-0.01 μg/ml for BR [3]. This fact suggests that throughout the length of the chemosensitive afferent fibers of dorsal-root origin there is a substrate which is responsible for the ability of these fibers to be depolarized by AC and BR. The action of AC and BR in low concentrations on the terminals ends with the generation of spreading potentials, whereas their action on dorsal root fibers is limited to prolonged depolarization, expressed as sensitization to the depolarizing action of potassium ions. This difference between the central and terminal portions of the afferent fibers is evidently purely quantitative, for with an increase in the AC and BR concentration the fibers of some animals also generate spreading potentials, as is shown by the appearance of pressor reflexes.

AC in concentrations of $10^{-8}$-$10^{-5}$ g/ml is known to be able to cause depolarization of efferent nonmyelinated fibers of the cervical sympathetic nerve [7, 8], without, however, causing the generation of action potentials. These arise only in response to the action of AC (not less than 1-50 μg/ml) on the preterminal segments of the sympathetic postganglionic fibers [6, 9, 12-14]. Since the sympathetic postganglionic fibersare nonmyelinated, by analogy it can be suggested that the afferent fibers depolarized by AC are also nonmyelinated. If this is so, the results of these experiments demonstrate a special property of the membrane of the afferent nonmyelinated fibers: that of prolonged preservation of the state of depolarization induced by AC and BR, even in very low concentrations.

In view of Sonina and Khayutin,[4], the sensitizing action of BR may be connected with the hyperalgesia arising in inflammation. A detailed analysis of the results on this question has been given elsewhere [4]. Here it will simply be mentioned that in a focus of inflammation there is, first, activation of proteases, and the formation of kinins [11, 16] and, second, an increase in the potassium ion concentration to 7-12 mM [17]. This last value is close to the threshold KCl concentration for excitation of nerve fibers sensitized by the action of BR. This result suggests that the sensitization phenomenon may lie at the basis of the hyperalgesia in inflammation and, in particular, in acute radiculitis.

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