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Further evidence for the release of noradrenalin under nerve stimulation and its effect on the potential difference in a toad nerve-skin preparation

Previous investigations by several authors[1–3] have demonstrated variations of the potential difference and short-circuit current across isolated toad skin upon stimulation of the nerve fibers which innervate it, or upon administration of adrenalin or noradrenalin at physiological concentrations to the vascular surface of the skin.

The extraordinary similarity of the variation profiles obtained by electrical stimulation of the nerve fibers and by the administration of adrenalin or noradrenalin suggests that the chemical mediator liberated during nervous stimulation might be sympathomimetic in nature.

In a previous report[3] it was shown that the effect of electrical stimulation is considerably decreased or disappears when the experimental animals are previously treated with reserpine. SÁNCHEZ, GONZÁLEZ AND CONCHA[3], studying the action of several chemical mediators, found that the only mediator whose effect closely resembled that of an electrical stimulation was noradrenalin, which supports the explanation of variations of potential difference being caused by the action of a sympathetic mediator.

The purpose of this article is to present more direct evidence that supports the hypothesis that the hyperpolarization effect and the short-circuit current increase obtained are due to the stimulation of sympathetic fibers which liberate noradrenalin at their terminals and that the noradrenalin is responsible for the aforementioned changes.

The investigations described here were conducted on the toad, *Bufo spinulosus*, employing a piece of skin, isolated from the foreleg, which was innervated by a brachial nerve branch. In each experiment simultaneous trials were carried out on preparations isolated from the two forelegs of the same toad.

The methods used for the mounting of the skin in the lucite chamber and for the measurements of the potential difference and the short-circuit current are those described by GONZÁLEZ, SÁNCHEZ AND CONCHA[2]. The nerve was electrically stimulated for a period of 5 min, using pulses having a potential of 10 V, a duration of 1 msec, and a frequency of 10 sec^{-1}. During the stimulation period (5 min) the nerve was placed under vaseline to prevent its desiccation. The noradrenalin was added in small volumes (approx. 0.01 ml) to the solution that bathed the vascular surface of the skin. The electrical and chemical stimuli were applied after allowing the potential difference and short-circuit current (measured simultaneously) to stabilize, which usually occurred after the skin had been mounted in the chamber for approximately 40 min. In the treatments with guanethidine sulphate, a longer period (120 min) was allowed to elapse in order to allow the guanethidine to have its maximum effect.

One group of toads was subjected to unilateral sympathectomy of the ganglia in the thoracic and cervical regions. These animals were maintained for 8 days after the operation to permit the total degeneration of the sympathetic fibers. The animals were then sacrificed and two nerve-skin preparations were isolated from each toad, one from the sympathectomized and one from the inoperated side.

Fig. 1a shows the result of simultaneous electrical stimulation of both the

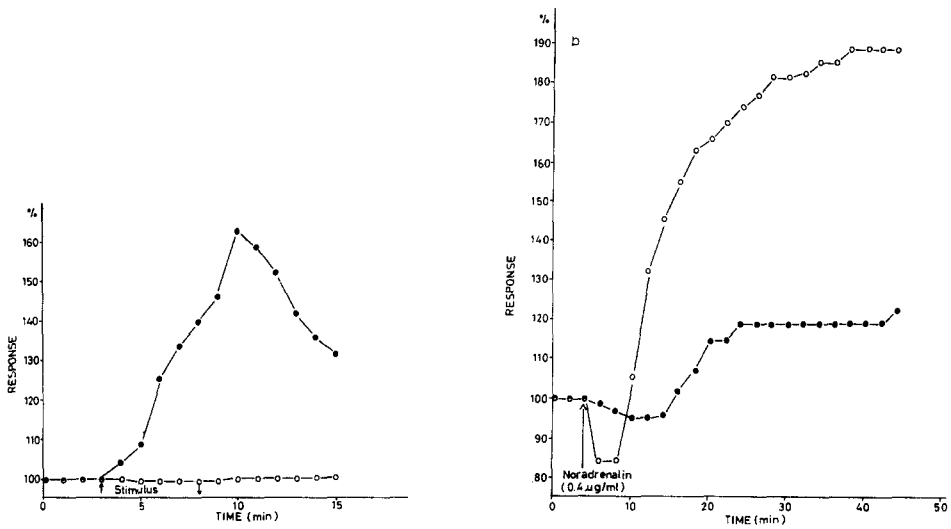

Fig. 1a. Effect of electrical stimulation of the brachial nerve branch on the potential difference expressed as per cent response. ○—○, sympathectomized side; ●—●, control side. Two preparations from the same toad (sympathectomized and control) were examined simultaneously.

Fig. 1b. Effect of L-noradrenalin (0.4 µg/ml) in the internal compartment of the lucite chamber on the potential difference expressed as per cent response. ○—○, sympathectomized side; ●—●, control side. Two preparations from the same toad (sympathectomized and control) were examined simultaneously.

sympathectomized and the control preparations. A normal response of about 80% hyperpolarization was observed in the control, while the sympathectomized side showed very little hyperpolarization; in other trials this was so reduced that the effect disappeared.

In other experiments, using the same type of preparations as those described above, chemical instead of electrical stimuli were applied by adding 0.4 µg/ml noradrenalin–HCl to the solutions bathing the vascular surface of the skin (both control and sympathectomized). In the sympathectomized preparation, as shown in Fig. 1b, this produced a depolarization and an exaggerated hyperpolarization in comparison to the response of the control side, thereby showing that the sympathectomized side developed a marked hypersensitivity to the chemical mediator.

The effect of catecholamine-depleting drugs, *e.g.*, guanethidine sulphate ("Ismeline", Ciba), was investigated in another group of toads with the sympathetic nervous system intact, by treating a nerve-skin preparation from one foreleg with the drug while using the preparation from the other foreleg as a control. Guanethidine was chosen for the experiment since it acts very well *in vitro* within a relatively short period of time (90 min) and it acts as a catechol depletor without affecting the effector. The guanethidine sulphate ($2 \cdot 10^{-5}$ g/l) was dissolved in the Ringer's solution that bathed the vascular surface of the skin. Fig. 2 shows that it completely blocked the effect of electrical stimulation while a normal response occurred on the control side.

In other experiments, with nerve-skin preparations previously treated with guanethidine in which the contact time was increased to 150 min, a hypersensitivity was observed upon stimulation with noradrenalin.

The short-circuit current and the potential difference were measured simultaneously in the majority of the experiments described here. The profiles of both were quite similar.

A small response was elicited by the electrical stimulation in several of the preparations isolated from sympathectomized animals, as in the case shown in Fig. 1a. This can possibly be due to an incomplete sympathectomy or a partial degeneration of the fibers. Other authors[8,9] have indicated that a period longer than 8 days is necessary for total degeneration. Nevertheless, there is no doubt that the sympathetic nervous system was responsible for the effects elicited by electrical stimulation.

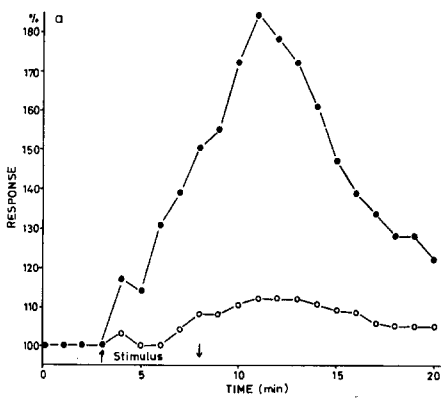

Fig. 2. Effect of electrical stimulation of the brachial nerve branch on the potential difference expressed as per cent response. ○—○, side previously treated (90 min before) with guanethidine sulphate; ●—●, control side. Two preparations from the same toad (guanethidine-treated and control) were examined simultaneously.

It is known that after a while the degenerated nerves develop a hypersensitivity to the chemical mediators that are liberated at their nerve endings. This evidently occurred in our experiments, as can be seen in Fig. 1b.

If it is supposed that the stimulation of sympathetic fibers releases a chemical mediator (noradrenalin) at their endings and the mediator acting upon epithelial effectors causes the described hyperpolarization, then this supposition can be strongly supported by demonstrating that after the elimination of the chemical mediator at the nerve endings there is no change of potential difference evoked by an electrical stimulation. This can be clearly seen in the case of Fig. 2, where guanethidine, a catecholamine-depleting drug, was used.

The evidence presented here, in addition to that presented by the authors in previous publications[2,3], permits us to postulate that the effect obtained by nervous electrical stimulation is due to the activation of sympathetic fibers which would liberate noradrenalin at their endings and that it is this noradrenalin which acts upon the transport epithelia of the skin causing the changes in potential difference and short-circuit current.

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Nuclear spin resonance evidence for complexing of sodium in frog skin

When frog skin is mounted as a flat sheet between 2 lucite chambers containing Ringer solution, a net movement of Na^+ from the outer to the inner side is observed even if the Na^+ concentration in the outside bathing solution is as dilute as 1–10 mM (refs. 1–4). Recent studies by ROTUNNO, POUCHAN AND CEREIJIDO[4] have indicated that, under these conditions, the concentration of Na^+ in the cells is about 97 mM. Under the short-circuit conditions that they have used to measure the net Na^+ flux, there exists an electrical potential difference of 15–30 mV between the outer solution and the cell (negative pole). However, this electrical potential difference is too small to explain the asymmetry of Na^+ distribution across the outer facing membrane of frog skin. Since there is no evidence that an active mechanism is operating at the outer cell border, ROTUNNO, POUCHAN AND CEREIJIDO[4] suggested the possibility that the Na^+ in the cell was contained in 2 different compartments, one of them being directly involved in Na^+ transport. This suggestion is strongly supported by recent studies carried out with Ringer solution with 1–10 mM Na^+, in which, in 90 min, only 37% of the Na^+ in the cells exchanged with the $^{22}Na^+$ in the Ringer solution ($^{22}Na^+$ flux across the frog skin equilibrated in less than 30 min*). With respect to the nature of the 2 compartments there are 2 main possibilities: (a) Physical compartments, where Na^+ is contained in 2 different spaces, one of them surrounded by a Na^+-impermeable barrier. These compartments might be represented, for instance, by Na^+ contained in different kinds of cells or cell organelles. (b) Chemical compartments, where Na^+ is contained in possibly the same physical compartments but in 2 different states. These compartments might be represented by free and bound Na^+. To study

* M. CEREIJIDO AND C. A. ROTUNNO, unpublished observations.