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PHARMACOLOGICAL EVIDENCE FOR THE INVOLVEMENT OF Na^+ CHANNELS IN THE RELEASE OF CATECHOLAMINES FROM PERFUSED ADRENAL GLANDS

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Veratridine (0.1 mM) was found to be effective in producing an increase in the catecholamine output from perfused guinea-pig adrenal glands in the presence of high concentrations of hexamethonium (1.83 mM) and atropine (28.8 μM). The response to veratridine was abolished by removal of either Na^+ or Ca^{2+} from perfusion media and by the addition of tetrodotoxin (0.1 μM). It is suggested that the response to veratridine may be due to an increase in the tetrodotoxin-sensitive Na^+ permeability of chromaffin cell membranes.

Introduction Quite recently, the ability of cultured adrenal chromaffin cells to evoke action potentials has been reported by two groups of investigators (Biales, Dichter & Tischler, 1976; Brandt, Hagiwara, Kidokoro & Miyazaki, 1976). According to them, the action potentials were mainly due to an increase in the tetrodotoxin (TTX)-sensitive Na^+ permeability of chromaffin cell membranes which it was suggested, played a significant role in the release of catecholamine under normal conditions. In order to confirm this hypothesis, it is necessary to study whether or not an increase in the TTX-sensitive Na^+ permeability causes a parallel increase in the catecholamine output under more physiological conditions.

The present experiments were carried out to investigate this possibility in perfused adrenal glands, by the use of veratridine which is known to increase the Na^+ permeability of excitable membranes (Ohta, Narahashi & Keeler, 1973).

Methods Guinea-pigs weighing between 600–800 g were anaestheized with sodium pentobarbitone (40 mg/kg) intraperitoneally. Both adrenal glands were perfused by the aortic route and the effluent was collected from the caudal vena cava following the general procedure adopted for cat glands by Douglas & Rubin (1961) except that the rate of adrenal efflux was kept constant (0.4–0.5 ml/min) with a peristaltic pump. Catecholamines (noradrenaline and adrenaline together) were assayed by the fluorimetric method of Anton & Sayre (1962).

The standard perfusion medium (Locke's solution) contained (mM): NaCl 156, KCl 5.6, $CaCl_2$ 2.2, Na_2HPO_4–NaH_2PO_4 buffer (pH 7.1) 3 and glucose 10. In Ca^{2+}-free medium, $CaCl_2$ was omitted with or without the addition of $MgCl_2$ (4 mM). In Na^+-free medium, all Na^+ was replaced by isotonic sucrose (9.25%) and K_2HPO_4–KH_2PO_4 buffer was used instead of sodium phosphate buffer. Total K^+ concentration was adjusted to 5.6 mM with KCl. All the media contained hexamethonium (1.83 mM) and atropine (28.8 μM). Perfusion media containing veratridine (0.1 mM) were prepared by the addition of a stock solution of the drug in dimethylsulphoxide. The final concentration of the solvent was 0.1% which had no effect on catecholamine output. The solutions were equilibrated with pure O_2 and perfusion was carried out at room temperature (approx 25°C).

Results Perfusion of the adrenal glands with medium containing veratridine (0.1 mM) caused an increase in the catecholamine output which reached a maximum during the period from 5 to 10 min and then gradually declined (Figure 1a). In Ca^{2+}-free medium, veratridine failed to increase the catecholamine output. Restoration of Ca^{2+} to the perfusion medium after the removal of veratridine caused the release of catecholamine (Figure 1b). Veratridine was also ineffective in producing the response in Na^+-free solution, although the restoration of Na^+ after the removal of veratridine caused increases in catecholamine output (Figure 1c).

The addition of veratridine for periods of 3 min at approximately 1 hourly intervals caused an increase in the catecholamine output at least three times in the same preparations, although the magnitude of the responses gradually decreased. The maximum values of each response were 662 ± 67 ($n = 6$), 350 ± 47 and 204 ± 43 ng/5 min ($n = 4$, mean ± s.e.) respectively. When the adrenal glands were perfused with the medium containing TTX (0.1 μM) 5 min before and

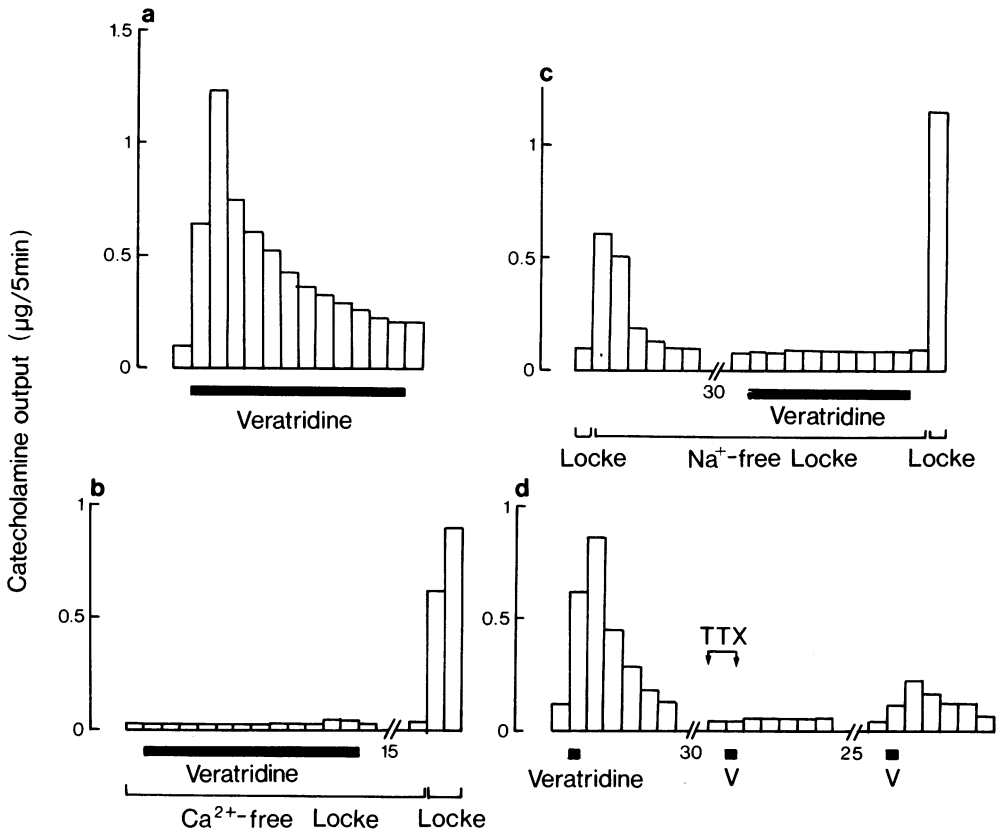

Figure 1 Effects of veratridine on the catecholamine output from perfused guinea-pig adrenal glands. (a) Typical response of catecholamine output induced by veratridine which was perfused with Locke. The lack of responses to veratridine in the absence of (b) Ca^{2+} ($n = 3$) and (c) Na^+ ($n = 3$) ions in the perfusion media. The first few responses in (c) resulted from exposure to the Na^+-free solution. The last responses in (b) and (c) were the result of changing to perfusion with standard Locke solution, thus replacing Ca^{2+} and Na^+. (d) Reversible inhibition by tetrodotoxin (TTX, 0.1 μM, $n = 4$) of the response induced by application of veratridine for 3 minutes. Veratridine (V,0.1 mM) was present for the period indicated by thick horizontal bars or squares. All the perfusion solutions contained hexamethonium (1.83 mM) and atropine (28.8 μM). Where the records are discontinous, the period (min) omitted is indicated by the numbers below the interruption. Each column shows the total catecholamine output (μg/5 min) that appeared in the adrenal effluent collected for 5 minutes.

during the second addition of veratridine, the response to veratridine was completely, but reversibly inhibited (Figure 1d).

Discussion In cultured adrenal chromaffin cells, acetylcholine (ACh) or electrical current evoked action potentials which were blocked by TTX and thus were due mainly to an increase in Na^+ permeability (Biales *et al.*, 1976; Brandt *et al.*, 1976). Veratridine is known to increase the TTX-sensitive Na^+ permeability of squid and crayfish giant axons (Ohta *et al.*, 1973). Thus veratridine may be useful in proving an involvement of Na^+ mechanisms in the secretory system of the adrenal medulla.

In the present experiments, veratridine caused an increase in the catecholamine output in the presence of hexamethonium and atropine. The response depended on both extracellular Ca^{2+} and Na^+ ions and was completely blocked by TTX. Veratridine elicited the TTX-sensitive release of noradrenaline from the peripheral adrenergic neurones (Thoa, Wooten, Axelrod & Kopin, 1975). Such an effect of veratridine in the adrenal chromaffin cells and adrenergic neurones is interesting, because the chromaffin cell membranes, like some excitable membranes, may have a Na^+ mechanism. Veratridine may cause depolarization of the adrenal chromaffin cells, resulting from a selective increase in the resting Na^+ permeability

(Ohta *et al.*, 1973). Action potentials evoked by ACh in some cultured chromaffin cells might be generated by this Na^+ mechanism.

However, there were some differences between the effects of ACh and veratridine. In a Na^+-free environment, ACh still caused the release of catecholamine (Douglas & Rubin, 1963). Furthermore, we have found that a part of the response to ACh remained in the presence of TTX (1 μM) (unpublished results). These results suggest that some mechanisms other than a Na^+ mechanism are involved in the effect of ACh. According to Brandt *et al.* (1976) action potentials in the chromaffin cell also have a Ca^{2+} component. Differences between the effects of ACh and veratridine may be explained by differences in ability to activate this Ca^{2+} component.

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