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BLOCKADE OF DESENSITIZATION OF NICOTINIC RECEPTORS OF THE CAT ADRENAL MEDULLA BY CONCANAVALIN A

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1 The possibility of concanavalin A (Con A) blocking the development of desensitization of nicotinic receptors of the cat adrenal gland has been investigated.

2 During perfusion of the adrenal gland with Krebs-bicarbonate solution containing acetylcholine (ACh), the rate of catecholamine (CA) secretion was very high in the first 2 min; thereafter, as perfusion with ACh was continued the output fell, to reach about 20% of the initial value in 10 minutes. When the adrenal gland was pretreated with Con A, the subsequent desensitization of release during continued infusion of ACh was prevented.

3 When the adrenal gland was perfused with high K^+ solution, there was always a large initial secretion of CA, and as perfusion with high K^+ continued the output fell, to reach about 15% of the initial rate in 10 minutes. Con A did not affect the rate of CA secretion induced by high K^+.

4 It is tentatively suggested that Con A blocks the desensitization of CA secretion evoked by ACh by interaction with the glycoprotein moiety of the nicotinic receptor of adrenal chromaffin cells.

Introduction

During perfusion of the cat adrenal gland with Locke solution containing acetylcholine (ACh) the rate of secretion of catecholamine (CA) overflowing into the venous perfusate is initially very high; however, as perfusion with ACh continues, the output declines progressively and falls to about 10% of the initial rate within a few minutes (Douglas & Rubin, 1961). Under appropriate experimental conditions, washout of the agonist leads to partial recovery of sensitivity, although some fall-off in CA release persists in successive tests with ACh. The cause of this fading of the response, which apparently is the result of desensitization of the adrenal nicotinic receptors to ACh, has not been well understood. The present investigation was undertaken to explore the mechanism by which desensitization of ACh-induced CA secretion from the adrenal medulla occurs. In these experiments we have used a specific glycoprotein binding agent, Concanavalin A (Con A, see Nicolson, 1974), on the assumption that the adrenal nicotinic receptor has a glycoprotein component that is involved in some way in the desensitization of nicotinic receptors.

Methods

Experiments were carried out with acutely denervated left adrenal glands of male cats. Cats (about 2 kg) were anaesthetized with ether, followed by chloralose (60 mg/kg, i.v.). The arrangements for the perfusion of the adrenal gland *in situ* were similar to those previously described (Dixon, Garcia & Kirpekar, 1975). The adrenal was perfused with Krebs-bicarbonate (Krebs) solution at room temperature (26–28°C) by means of a pump (Sigmamotor, Model A14E). The Krebs solution was bubbled with a mixture of 95% O_2 and 5% CO_2; the final pH was 7.4 to 7.5.

The composition of Krebs solution was as follows (mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4.7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11. The secretion of CA was evoked by perfusion of the gland with either Krebs solution containing ACh chloride (55 μM) or a potassium-rich solution (90 mM) for 10 minutes. The composition of potassium-rich solution was as follows (mM): K_2SO_4 45, NaCl 56.2, $CaCl_2$ 2.5, $MgSO_4.7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11. Samples were collected from the adrenolumbar vein, each for 2 minutes. Each secretory response was obtained from a different prep-

aration, and each type of experiment was repeated at least four times. CA content of the venous perfusates was determined fluorometrically (Anton & Sayre, 1962), without the intermediate alumina adsorption procedure. CA values are expressed as noradrenaline equivalents.

Concanavalin A (Con A, Sigma, Grade IV) was dissolved in Krebs solution to give a final concentration of 1 μM.

The adrenal gland was initially perfused with normal Krebs solution for about 30 minutes. A 2 min control sample was collected, to determine the background activity. The gland was then perfused with Krebs solution containing ACh or high K^+ for 10 min, followed by normal Krebs solution for an additional 30 minutes. In other experiments the gland was perfused with Krebs solution containing Con A for 60 min and then, in the presence of Con A, ACh or high K^+ was infused for 10 min as before.

Results

After perfusion with Krebs solution for 30 min, the resting secretion of CA was about 0.05 μg min^{-1} per gland. Figure 1a shows that during perfusion with ACh (55 μM) the rate of CA secretion was increased greatly during the first 2 min and as perfusion with ACh was continued, the outputs progressively declined, to reach about 20% of this initial rate within 10 minutes. Even though the CA output during the first 2 min of ACh infusion varied in different glands, the subsequent desensitization of secretion during ACh perfusion followed the same pattern as described above. When the gland was perfused with Con A (1 μM) resting secretion of CA was not appreciably different from the glands perfused with Krebs solution alone. Even though Con A did not appreciably modify the initial secretory response to ACh, it almost completely blocked the subsequent desensitization of release as perfusion with ACh continued. In the experiment shown in Figure 1b, the adrenal gland was perfused with Con A for 1 h and then challenged with ACh in the presence of Con A. CA output during the first 2 min of ACh plus Con A infusion was also greatly enhanced, as from untreated glands. However, as perfusion with this solution was continued, the CA output during the subsequent collection periods did not fall but actually rose to about 115% of the initial output during the final 2 min perfusion period. In 6 experiments, the output during the final 2 min of ACh infusion was 130 ± 14% of the initial 2 min output. In one experiment, ACh plus Con A was infused for 20 min, and the CA output during the final collection period (18 to 20 min) was still comparable to the initial 2 min output. The blockade of desensitization by Con A was slowly reversible, since after perfusion for 1 h with Krebs solution without Con A,

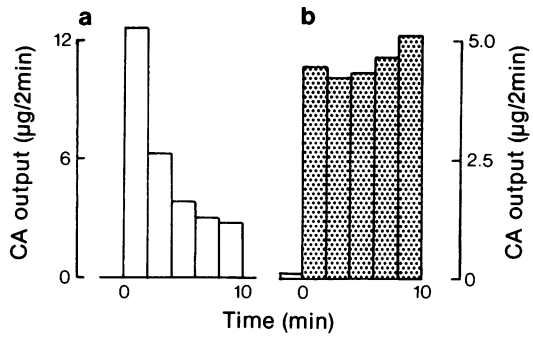

Figure 1 Blockade of densitization of catecholamine (CA) release by concanavalin A (Con A). Cat adrenal glands were perfused with Krebs-bicarbonate solution. (a) After 30 min perfusion with Krebs solution, a control sample was collected to determine the background activity (shown by the base line, 0.02 μg/2 min); perfusion was then changed to Krebs solution containing acetylcholine (ACh, 55 μM) and successive 2 min samples were collected. (b) Adrenal gland was perfused with Con A (1 μM) solution for 1 h and control sample was collected to determine the background activity (shown by the open column, 0.1 μg/2 min). Perfusion was then changed to Krebs solution containing Con A plus ACh, and successive 2 min samples were taken. Each response was obtained from a different preparation.

the secretory response to ACh only partially recovered and the output during the last 2 min collection period was still about 40–50% of the initial output.

Con A blockade of desensitization of CA release was specific only to release induced by stimulation of cholinoceptors, since it did not affect the rate of CA secretion induced by infusion of high K^+. When the adrenal gland was perfused with high K^+ solution (90 mM), as with ACh there was always a large increase in CA secretion, and as perfusion with high K^+ was continued the output fell, to reach about 15% of the initial rate within 10 min (Figure 2a). However, during perfusion with Con A the secretory response to high K^+ remained comparable to the control response, and the desensitization of CA release induced by high K^+ was not prevented by Con A (Figure 2b).

Discussion

Although the phenomenon of desensitization to nicotinic agents in the adrenal medullary cells has been known for some time (Douglas & Rubin, 1961), it has not been studied as extensively as the desensitization to these agonists at the neuromuscular junction

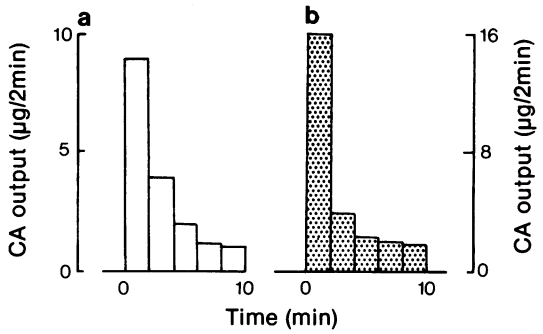

Figure 2 The effect of concanavalin A (Con A) on the time course of release of catecholamine (CA) during continuous perfusion of the adrenals with potassium-rich solution (90 mM). (a) Background activity of the control sample is shown by the base line (0.1 µg/2 min); perfusion was then changed to potassium-rich solution, and successive 2 min samples were collected. (b) Adrenal gland was perfused with Con A (1 µM) solution for 1 h; the background activity of the control samples was negligible, and is shown by a thin line at the beginning of the record. Perfusion was then changed to potassium-rich solution containing Con A, and successive 2 min samples were taken. Each response was obtained from a different preparation.

and at autonomic ganglia (see Hubbard, 1973; Haefely, 1972). It has been shown, both for the neuromuscular junction and autonomic ganglia, that during the development of desensitization the postsynaptic membrane repolarizes, despite the presence of the nicotinic agonists. Because of the common developmental origins between autonomic ganglia and the adrenal medulla, it is quite possible that when adrenal medullary cells are exposed to ACh they may also undergo similar changes, i.e., an initial secretory response associated with depolarization and a subsequent desensitization of release, even in the continued presence of the agonist, with repolarization of adrenal chromaffin cells.

We have shown in this paper that Con A successfully blocked the subsequent desensitization of CA release induced by stimulation of the nicotinic and muscarinic cholinoceptors of the adrenal medullary cells with ACh. It was remarkable that when the adrenal gland was treated with Con A, the rate of CA secretion was sustained throughout the infusion of ACh. Even though it is known that ACh causes secretion of CA from the cat adrenal gland by stimulating both nicotinic and muscarinic receptors (Douglas & Poisner, 1965; Rubin & Miele, 1968), the contribution of the muscarinic receptors to the total secretory response is only minimal, and of doubtful physiological significance (Douglas, 1975; Wilson & Kirshner, 1977). We have therefore assumed in the present experiments that the secretory response to ACh and the blockade of desensitization of release by Con A are primarily due to the interaction of these agents with nicotinic receptors. We cannot at present rule out the contribution of muscarinic receptors to the total secretory response to ACh. It is unlikely that Con A significantly affects membrane properties of the chromaffin cells, since it did not cause secretion of CA by itself, or appreciably affect the initial secretory response to ACh; furthermore, it did not affect the response to potassium-rich solution, which is known to have a direct stimulant effect on the chromaffin cells of the adrenal medulla (Vogt, 1952). It appears, therefore, that Con A must interfere in some way with the subsequent desensitization of release by interacting with the nicotinic receptors.

Mathers & Usherwood (1976) showed that Con A selectively blocks desensitization of only one type of extrajunctional glutamate receptor of the locust muscle fibre without affecting the other glutamate receptors. It would be interesting, therefore, to know, whether Con A blocks desensitization of other nicotinic receptors, for example those located on sympathetic nerve endings in sympathetically innervated organs (see Muscholl, 1970), and whether it would prevent desensitization of the muscarinic receptors of the cat adrenal gland, if the phenomenon is exhibited also by them.

A number of models have been advanced to explain the phenomenon of desensitization of nicotinic receptors (see Rang & Ritter, 1970). According to the most widely accepted model, based on electrophysiological studies of the endplate of the frog neuromuscular junction, it is believed that the nicotinic receptors, after combining with the agonist, exist in at least two stable forms, representing the active (sensitive) state and the inactive (desensitized) state (Katz & Thesleff, 1957). If the nicotinic receptors of the adrenal medulla after their reaction with ACh also exist in the active and inactive conformations, then Con A may block desensitization of release by blocking the transition of the nicotinic receptors from the activated to the inactivated state. Since Con A binds to receptor glycoproteins, it is tentatively suggested that these specific glycoprotein sites on the receptor—susceptible to blockade by Con A, are responsible for the desensitization of release.

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