Proc. Natl. Acad. Sci. USA
Vol. 76, No. 4, pp. 2081–2083, April 1979
Physiological Sciences

Release of catecholamines from perfused cat adrenal gland by veratridine

(acetylcholine/tetrodotoxin/secretion regulation)

S. M. KIRPEKAR AND J. C. PRAT

Department of Pharmacology, State University of New York Downstate Medical Center, Brooklyn, New York 11203

Communicated by Chandler McC. Brooks, January 2, 1979

ABSTRACT **Experiments were undertaken to verify the existence of fast sodium channels in the adrenal chromaffin cell membrane and to assess their contribution to the physiological release of catecholamines. We have used veratridine to activate sodium channels. Veratridine causes secretion of catecholamines from perfused cat adrenal gland. Secretory response to veratridine is calcium dependent and abolished by tetrodotoxin. Secretion of catecholamines by acetylcholine is only partially blocked by tetrodotoxin. It is concluded that the adrenal chromaffin cell membrane contains fast sodium channels directly comparable to those of impulse-propagating neurons, but they do not appear to be essential in the secretory response to acetylcholine or splanchnic nerve stimulation.**

It is generally believed that acetylcholine (AcCho)-induced secretion of catecholamines from the adrenal medulla occurs by depolarization of the chromaffin cells in a nonimpulsive manner. Intracellular recordings from chromaffin cells have shown that AcCho-induced depolarization is due to inward currents of sodium and calcium ions, with sodium contributing quantitatively more to depolarization (1). Even though inward sodium current plays a major role in the AcCho-induced depolarization, secretion of catecholamines by AcCho is obtained in sodium-free media (2).

Veratridine has been used extensively to open sodium channels in a number of tissues. Veratridine evokes repetitive action potentials in nerves and, at higher concentrations, sodium-dependent large depolarizations (3, 4). Veratridine also has similar effects on nonneuronal cells of the pancreatic β cell (5). Tetrodotoxin (TTX) abolishes veratridine-induced action potentials and large depolarizations (3, 4, 6). Because TTX selectively blocks the voltage-dependent "fast" sodium channels without interfering with slow sodium-dependent nonimpulsive electrical activity (7, 8) and because it abolishes the veratridine response (i.e., repetitive action potentials and large depolarizations), the main site of action of veratridine appears to be localized to the fast sodium channels.

The present experiments were undertaken to determine the existence of fast sodium channels in the adrenal chromaffin cell membrane and to assess their contribution to the physiological release of catecholamines by AcCho. We have used veratridine to induce secretion of catecholamines from perfused cat adrenal gland and TTX to block the veratridine response presumably mediated by activation of sodium channels.

METHODS

Experiments were carried out in acutely denervated cat adrenal glands. Cats (about 2 kg) were anesthetized with ether, followed by chloralose (60 mg/kg, intravenously). The adrenal was perfused *in situ* with Krebs/bicarbonate (Krebs) solution at room temperature (26–28°C) by means of a pump (9). The Krebs solution was equilibrated with a mixture of 95% O_2 and 5% CO_2; the final pH was 7.4–7.5.

The composition of Krebs solution was as follows (mM): NaCl, 119; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2 \cdot 6H_2O$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; and glucose, 11. Low-calcium solution contained 0.1 mM calcium. Secretion of catecholamines was evoked by perfusion of the gland with Krebs solution containing veratridine or AcCho for 2 min. Samples were collected from the adrenolumbar vein, each for 2 min. Catecholamine release induced by veratridine included the extra release over the control output during the 2 min of exposure to the drug and in the succeeding 8 min. AcCho response included the extra secretion during 2 min of infusion and in the succeeding 6 min. Over 80–90% of catecholamine release induced by AcCho occurred during the AcCho infusion. Each type of experiment was repeated at least four times. Catecholamine content of the venous perfusate was determined fluorometrically (10) without the intermediate alumina adsorption procedure. Catecholamine values are expressed as norepinephrine equivalents.

The adrenal gland was initially perfused with normal Krebs solution for about 30 min. A 2-min control sample was collected to determine the background activity. The gland was then perfused with Krebs solution containing veratridine or AcCho for 2 min, followed by normal Krebs solution. In order to study the effect of hexamethonium on veratridine-induced secretion, we first perfused the gland with Krebs solution containing 100 μM hexamethonium for 10 min and then with hexamethonium and veratridine for 2 min. TTX was infused simultaneously with veratridine or AcCho. In estimating the inhibitory effect of TTX, hexamethonium, and low calcium treatment on veratridine- or AcCho-induced release, the spontaneous decline in catecholamine output on repeated applications of these agents was considered.

RESULTS

Effect of Veratridine on Release of Catecholamines. After perfusion with Krebs solution for 30 min, the resting secretion of catecholamines was 0.32 μg min^{-1} per gland. As the perfusion of the gland continued, the resting secretion was further suppressed to 0.035 μg min^{-1}. However, resting secretion varied from practically nothing to about 0.4 μg min^{-1} in different preparations. Fig. 1 shows that catecholamines release was minimal at 1 μM veratridine and, as the concentration was increased from 1 to 30 μM, greater release was obtained. Catecholamine release was about 25-fold greater at 30 μM than at 3 μM. In contrast to the release of catecholamines induced by AcCho, the release induced by the highest concentration of veratridine used appeared to be only slowly reversible. In experiments in which the adrenals were perfused with veratridine

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: AcCho, acetylcholine; TTX, tetrodotoxin; Krebs solution, Krebs/bicarbonate solution.

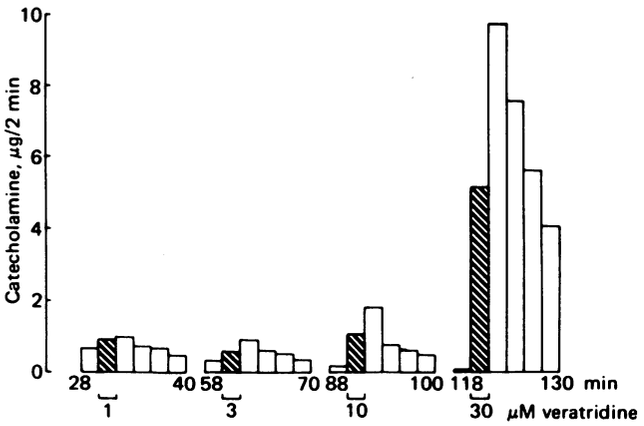

FIG. 1. Effect of different concentrations of veratridine on catecholamine release from perfused cat adrenal gland. In this and subsequent figures, each bar represents the catecholamines found in a 2-min collection period. Hatched bars show output in response to infusions of different concentrations of veratridine. Note that secretory response to veratridine is only slowly reversible. Open bars show catecholamine outputs during perfusion with Krebs solution.

(30 μM), the rate of catecholamine secretion was not only increased during the 2-min perfusion of the drug, but remained high for at least an additional 8 min during subsequent perfusion with drug-free Krebs solution. Thus, depolarization of chromaffin cells presumably accounts for the prolonged secretory response to veratridine since this effect was still evident even after washout of the drug. On repeated infusions, the secretory response to veratridine was greatly reduced. If the glands were challenged with veratridine at intervals of 20–30 min for a second and a third time, the outputs were 64 ± 5 and 38 ± 5.6% (±SEM), respectively, as compared to the outputs obtained during the first stimulation.

In order to compare the rate of release by veratridine with that by other stimuli, we evoked catecholamine release by an infusion of AcCho. The release induced by veratridine (30 μM) was about 2- to 4-fold greater than the release obtained by an infusion of AcCho (55 μM).

Effect of TTX on Release of Catecholamines by Veratridine. Since 30 μM veratridine was effective in releasing substantial quantities of catecholamines, we have used only this concentration in this and in the following series of experiments. TTX (0.6 μM) does not affect the spontaneous release of catecholamines, yet it almost completely blocked the release induced by veratridine (Fig. 2). Similar blockade of release was obtained in five experiments (0.4 ± 0.4% of the initial output). On reperfusion with normal Krebs solution for another 30 min, the veratridine response partially recovered. Recovery varied in different experiments, and in five experiments it amounted to 35 ± 12% of the initial response.

Because veratridine produced repetitive action potentials and, at high concentrations, large depolarizations, it is possible that the veratridine response might conceivably have been due to release of AcCho from the splanchnic nerves contributing to catecholamine release. In order to test this possibility, we studied veratridine-induced release in the presence of hexamethonium. In four experiments hexamethonium (100 μM) reduced the veratridine response by 24 ± 8.8%. When the glands were subsequently perfused with hexamethonium plus TTX, the response to veratridine was entirely suppressed. Because hexamethonium partially blocked the veratridine response, a portion of the catecholamine release may indeed have been due to release of AcCho. However, the remainder of the veratridine response must be attributed to the direct action of veratridine on chromaffin cell membranes.

In one experiment the adrenal gland was chronically denervated about a week before the experiment. Infusion of veratridine (30 μM) caused secretion of catecholamines, but the response appeared to be reduced by about 30% as compared to the response obtained from the innervated glands.

Effect of Calcium on Release of Catecholamines by Veratridine. Fig. 3 shows that the veratridine-induced secretion of catecholamines was entirely dependent on the presence of calcium in the perfusion medium. Secretory response to veratridine (30 μM) was first obtained. During perfusion of the gland with Krebs solution containing 0.1 mM calcium, the release was almost completely blocked. The mean output in calcium-free solution was 0.75 ± 0.47% (n = 4) of the initial release. On reperfusion with normal Krebs solution, the response to veratridine was partially restored. In four experiments, the mean recovery was 44 ± 1% of the initial release. These experiments suggest that the veratridine-induced release is entirely dependent on the presence of calcium in the perfusion medium and that intracellularly bound calcium does not compensate for lack of calcium entering from the outside. The calcium dependence of the veratridine response in the adrenal gland is quite different from its secretory response in the pancreas. Veratridine evokes insulin release from the pancreatic β cells in calcium-free medium (5). The difference in the calcium dependence of the veratridine response probably reflects the size of intracellular calcium stores in the two tissues.

Effect of TTX on Release of Catecholamines by AcCho. Because secretion of catecholamines by AcCho has been shown to occur in sodium-free solution (2), it was of some interest to study the effect of TTX on AcCho-mediated release. Repeated infusions of AcCho (55 μM) at intervals of about 20–30 min resulted in a 25% reduction in catecholamine output from the

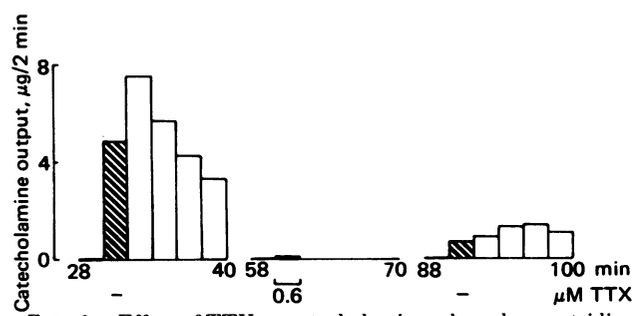

FIG. 2. Effect of TTX on catecholamine release by veratridine. Background release was 0.01 μg min^{-1}. Hatched bars show catecholamine output in response to veratridine infusion (30 μM). TTX (0.6 μM) was infused for 2 min along with veratridine (*Middle*). Open bars show catecholamine outputs during perfusion with Krebs solution.

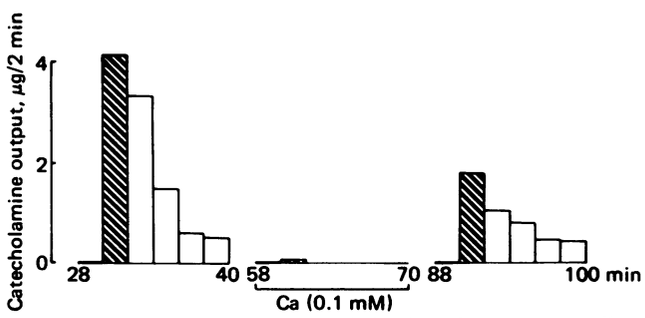

FIG. 3. Effect of calcium on catecholamine release by veratridine. Background release was 0.02 μg min^{-1}. Hatched bars show catecholamine output in response to veratridine (30 μM). Adrenal was perfused with low calcium solution (0.1 mM) from 58 to 70 min. Open bars show catecholamine outputs during perfusion with Krebs solution.

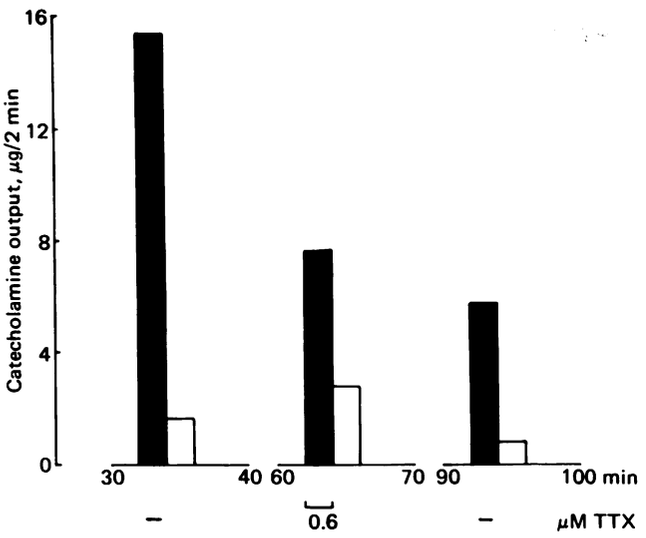

FIG. 4. Effect of TTX on catecholamine release by AcCho. Background release was zero. Filled bars show catecholamine output in response to AcCho (55 μM). TTX (0.6 μM) was infused for 2 min along with AcCho (*Middle*). Open bars show catecholamine outputs during perfusion with Krebs solution.

preceding response. Fig. 4 shows that TTX (0.6 μM) blocked the AcCho (55 μM) response by about 20% as compared to the expected AcCho response. In five experiments, TTX blocked the AcCho response by 28 ± 7%. When the TTX concentration was increased to 3 μM, the blockade was not further enhanced.

DISCUSSION

We have shown that veratridine elicits a powerful secretory response from perfused cat adrenal gland and that TTX almost completely blocks it. Moreover, the veratridine response is entirely calcium dependent. TTX prevents depolarization in impulse-propagating neurons by specifically blocking only the fast sodium channels. Because it abolished the secretory response of the adrenal gland to veratridine, it might be argued that depolarization of chromaffin cells resulted from the passage of sodium ions through fast sodium channels, with the subsequent opening of the voltage-sensitive calcium channels to release catecholamines. Thus it appears that veratridine depolarized chromaffin cells in a manner very similar to depolarization of impulse-propagating neurons.

In contrast, TTX only partially blocks the secretory response to AcCho. We do not know whether the AcCho-mediated depolarization of the chromaffin cells is blocked by TTX, and it certainly would not be expected to block the calcium channel responsible for catecholamine secretion. Even though the membrane potential of chromaffin cells exposed to AcCho in a sodium-free solution shows significant depolarization (1), the identity of the ion(s) responsible for depolarization has not been clearly established (1, 11). Because AcCho partially depolarizes the membrane even in sodium-free solution, it is fair to assume that the cell membrane may also have undergone depolarization by AcCho in the presence of TTX. AcCho-induced depolarization is slow, presumably mediated through the nonimpulsive entry of sodium, and possibly calcium, into the chromaffin cell and hence probably not amenable to blockade by TTX. Irrespective of the ability of TTX to block the AcCho-induced depolarization, TTX does not block secretion and hence the AcCho-mediated voltage-dependent entry of calcium ions. In order to increase calcium permeability, AcCho, even in the presence of TTX, must first depolarize the cell membrane to open the calcium channels.

Our results suggest that secretion of catecholamines in response to veratridine or AcCho application is brought about by calcium entry through voltage-sensitive TTX-resistant calcium channels rather than through TTX-sensitive sodium channels. The difference lies in the manner in which both agents activate the TTX-resistant calcium channel. Veratridine, by activating the TTX-sensitive fast sodium channel, increases the intracellular positive charge that leads to depolarization and thus opens the voltage-sensitive calcium channel. AcCho produces depolarization by activating sodium or calcium channels or both, or at least an electrical disturbance even in the presence of TTX or sodium-free solution, and thus opens the same calcium channels. A prior depolarization by AcCho or veratridine seems to be a prerequisite to the subsequent opening of calcium channels. In conclusion, we suggest that the chromaffin cell membrane contains fast sodium channels directly comparable to those of impulse-propagating neurons, but they do not appear to be essential in the secretory response to AcCho or splanchnic nerve stimulation.

This work was supported by Grants 1 RO 1 HL 22170-01 from the National Institutes of Health and 412-2181-A from the New York Heart Association.

1. Douglas, W. W., Kanno, T. & Sampson, S. R. (1967) *J. Physiol.* **191**, 107–121.
2. Douglas, W. W. & Rubin, R. P. (1961) *J. Physiol.* **159**, 40–57.
3. Ohta, M., Narahashi, T. & Keeler, R. F. (1973) *J. Pharmacol. Exp. Ther.* **184**, 143–154.
4. Lowe, D. A., Bush, B. M. H. & Ripley, S. H. (1978) *Nature (London)* **274**, 289–290.
5. Donatsch, P., Lowe, D. A., Richardson, B. P. & Taylor, P. (1977) *J. Physiol.* **267**, 357–376.
6. Wellhömer, H. H. (1970) *Nauyn-Schmiedeberg's Arch. Pharmakol.* **267**, 185–188.
7. Narahashi, T. (1974) *Physiol. Rev.* **54**, 813–889.
8. Kao, C. Y. (1966) *Pharmacol. Rev.* **18**, 997–1049.
9. Dixon, W., Garcia, A. G. & Kirpekar, S. M. (1975) *J. Physiol.* **204**, 804–824.
10. Anton, A. H. & Sayre, D. F. (1962) *J. Pharmac. Exp. Ther.* **138**, 360–375.
11. Brandt, B. L., Hagiwara, S., Kidokoro, Y. & Miyazaki, S. (1976) *J. Physiol.* **263**, 417–439.