Br. J. Pharmac. (1979), **65**, 319–330

THE EFFECT OF VERATRIDINE ON THE RELEASE OF CATECHOLAMINES FROM THE PERFUSED ADRENAL GLAND

S. ITO, Y. NAKAZATO & A. OHGA

Department of Pharmacology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan

1 Experiments on perfused adrenal glands of guinea-pigs were carried out to study the catecholamine output induced by veratridine in the presence of hexamethonium and atropine.

2 Veratridine (10 μM to 200 μM) caused a dose-dependent increase in catecholamine output.

3 The addition of veratridine to the perfusion medium for a period of 3 min caused an increase in catecholamine output which reached a maximum 5 min to 10 min after withdrawal of the drug. The catecholamine output then gradually declined and reached near resting values within 30 minutes. It was never sustained for a longer period, even when veratridine was infused for 1 hour.

4 Veratridine failed to increase the catecholamine output in the absence of extracellular Ca^{2+}. However, the addition of Ca^{2+} after an infusion of veratridine (100 μM) in the absence of Ca^{2+} caused an increase in the catecholamine output which was proportional to the concentration of Ca^{2+} (0.55 mM to 8.8 mM) used.

5 Veratridine did not increase the catecholamine output in the absence of extracellular Na^+ ions, NaCl being replaced by equimolar choline chloride or LiCl. Veratridine also failed to evoke catecholamine output in a Na^+-free solution in which Na^+ was replaced by sucrose; this was the case even in the presence of a high concentration of Ca^{2+} (8.8 mM).

6 Tetrodotoxin (0.1 μM) and excess Mg^{2+} (20 mM) reversibly inhibited the catecholamine output induced by veratridine.

7 Ouabain (10 μM) significantly potentiated the veratridine-induced catecholamine output.

8 It is suggested that Na^+-dependent Ca^{2+} influx as well as voltage-dependent Ca^{2+} influx mechanisms may be involved in the catecholamine output induced by veratridine.

Introduction

Two groups of investigators reported that cultured adrenal chromaffin cells could produce action potentials and suggested that the catecholamine output induced by acetylcholine (ACh) was associated with generation of action potentials under normal conditions (Biales, Dichter & Tischler, 1976; Brandt, Hagiwara, Kidokoro & Miyazaki, 1976). An essential requirement for this hypothesis was that Na^+ channels were present in the membranes of adrenal chromaffin cells under normal conditions.

Veratridine has been found to be effective in increasing Na^+ permeability by decreasing the rate of inactivation of Na^+ channels (Ohta, Narahashi & Keeler, 1973). Therefore, veratridine together with tetrodotoxin (TTX) have proved to be useful tools for demonstrating an involvement of Na^+ mechanisms in the secretory function of adrenergic neurones (Thoa, Wooten, Axelrod & Kopin, 1975) and pancreatic β-cells (Donatsch, Lowe, Richardson & Taylor, 1977) and of synaptosomes separated from rat brain (Blaustein & Goldring, 1975). We have recently presented evidence for the involvement of Na^+-channels in the catecholamine release induced by veratridine from perfused adrenal glands and discussed the discrepancy between the effect of ACh and veratridine (Ito, Nakazato & Ohga, 1978).

It is well-known that Ca^{2+} is indispensable for stimulus-secretion coupling in adrenal chromaffin cells (Douglas, 1968; 1975). Furthermore, Ca^{2+} movement across chromaffin cell membranes was found to be affected by the Na^+ gradient (Aguirre, Pinto & Trifaró, 1977; Rink, 1977) as in squid axon membranes (Baker, Blaustein, Hodgkin & Steinhardt, 1969; Blaustein & Hodgkin, 1969; Baker, 1972; 1976). A study of the effect of veratridine may provide more information on the role of Na^+ ions in the catechol-

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amine secretion from adrenal chromaffin cells. The present experiments were carried out to investigate this problem in perfused adrenal glands.

Methods

Guinea-pigs weighing between 500 and 800 g were anaesthetized with sodium pentobarbitone (40 mg/kg) intraperitoneally. Both adrenal glands were perfused following the general procedure described for cat adrenal glands by Douglas & Rubin (1961). The glands were perfused at a flow rate of 0.4 to 0.5 ml/min by a peristaltic pump. The perfusion fluids were infused into the lower aorta through a polyethylene cannula and were collected from a polyethylene cannula inserted in the caudal vena cava. In guinea-pigs with a double vena cava, a cannula was inserted into each branch. After the beginning of the perfusion, the glands together with their surrounding tissues, including both kidneys, were isolated and transferred to a dissection plate. This procedure allowed the detection of any leakage of perfusion fluid from the preparation and the perfusion to continue for at least 3 hours. When leakage did occur and the ratio of inflow/outflow fell to less than 0.85, the experiment was discontinued. The introduction of drugs was started 30 to 60 min after the beginning of the perfusion.

The standard perfusion medium was Locke solution of the following composition (mM); NaCl 154, KCl 5.6, $CaCl_2$ 2.2, Na_2HPO_4-NaH_2PO_4 buffer (pH 7.1) 3 and glucose 10. Pure O_2 was continuously bubbled through this solution and the perfusion was carried out at room temperature (approximately 25°C). In Na^+-free solutions, NaCl was replaced by isotonic sucrose (9.25%), equimolar choline chloride or LiCl. In addition, Na-phosphate buffer was replaced by Tris aminomethane buffer (2.5 mM). In Ca^{2+}-free solution, $MgCl_2$ (4 mM) was used instead of $CaCl_2$. In solutions in which the concentration of Ca^{2+} varied between 0.55 mM and 8.8 mM, the Na-phosphate buffer was replaced by Tris aminomethane buffer (2.5 mM) in order to avoid the precipitation of Ca^{2+}. Solutions containing an excess of Mg^{2+} were prepared by addition of $MgCl_2$ (20 mM) without adjusting the tonicity. In solutions containing 56 mM KCl, the NaCl content was reduced correspondingly. All solutions contained hexamethonium (1.83 mM) and atropine (28.8 μM) to prevent indirect effects through the activation of both nicotinic and muscarinic receptors on the chromaffin cells.

Perfusion fluids containing veratridine were prepared by the addition of an appropriate quantity of a stock solution of the drug dissolved in dimethylsulphoxide. The final concentration of this solvent was less than 0.1% which had no effect on catecholamine output (Cochrane, Douglas, Mouri & Nakazato, 1975). The required quantities of ouabain and TTX were added to Locke solution from concentrated stock solutions.

The adrenal effluent was usually collected over a period of 5 min (sometimes for 2.5 or 10 min) into glass tubes kept on ice. All the samples were acidified with 8 N perchloric acid giving a final concentration of 0.4 N. The acidified samples were centrifuged at 25,000 *g* at 5°C for 10 minutes. The clear supernatants were transferred to small test tubes and stored on ice until assay. The assay of catecholamines (adrenaline) was performed by the fluorimetric method of Anton & Sayre (1962).

Results

Dose-dependent increase in the catecholamine secretion from perfused adrenal glands in response to veratridine

Veratridine caused a dose-dependent increase in the catecholamine secretion from the perfused guinea-pig adrenal gland. This effect of veratridine is probably due to a direct action of the drug on adrenal chromaffin cells, since the experiments were carried out in the presence of high concentrations of hexamethonium (1.83 mM) and atropine (28.8 μM) to inhibit indirect effects induced by ACh released from the splanchnic nerve terminals (Douglas & Poisner, 1965; Douglas, Kanno & Sampson, 1967a; Cochrane *et al.*, 1975).

Figure 1 shows that veratridine in concentrations of 10 μM to 200 μM caused a dose-dependent increase in the secretion of catecholamines, when infused for a period of 3 minutes. With concentrations ranging from 10 μM to 100 μM, the response to veratridine developed gradually and attained a maximum within 5 to 10 min after the start of the infusion of veratridine and then slowly declined. In contrast, veratridine at a concentration of 200 μM always caused a rapid increase in catecholamine output which reached a maximum during the first 5 minutes. Similar results were obtained in four other experiments. In all further experiments, we used 100 μM veratridine, a concentration that caused a submaximal effect.

Catecholamine release induced by brief perfusions with vertridine and excess potassium

Catecholamine secretion was first stimulated for 2 min by infusion of Locke solution containing an excess of K^+. This was followed by an infusion of normal Locke solution for 13 minutes. Locke solution containing veratridine was then infused for a period of 3 minutes. This sequence was repeated at 35 to 40 min intervals during which normal Locke solution

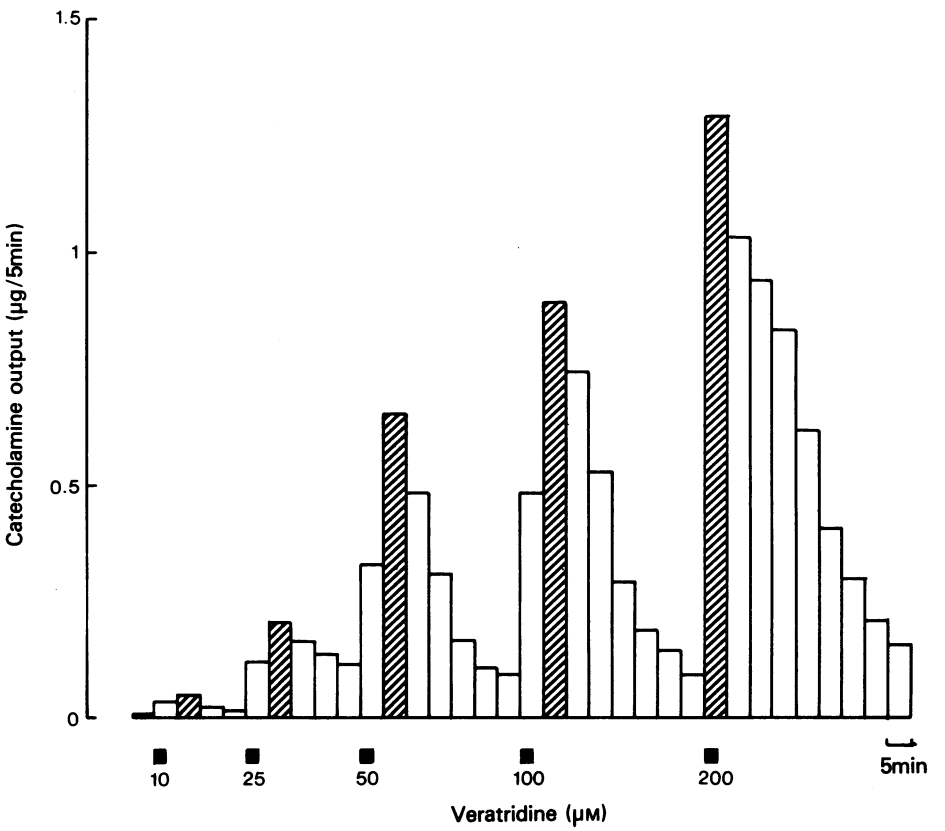

Figure 1 Dose-dependent increase in the catecholamine output induced by veratridine from perfused adrenal glands of guinea-pigs. Adrenal glands were repeatedly stimulated with veratridine for periods of 3 min at 20 to 40 min intervals during which the veratridine concentration was progressively increased from 10 to 200 μM. Filled squares (■) and the numbers below them indicate the length of the exposure and the concentration of veratridine. Hatched columns show the maximum output of catecholamines induced by each concentration of veratridine. In this and in all subsequent figures, the columns represent the total amount of catecholamines (μg) present in the adrenal effluent which was collected for 5 min (unless stated otherwise). Similar results were obtained in three other experiments.

was infused. The results obtained from seven experiments are summarized in Figure 2. As shown in this figure, veratridine and excess K^+ stimulated catecholamine secretion. The effect decreased on repetition.

The response to excess K^+ was almost terminated within the first 5 min collection period. On the other hand, the responses to veratridine developed gradually, attained a maximum during the second 5 min collection period and then slowly decreased over a period of about 30 minutes.

Catecholamine output induced by a prolonged exposure to veratridine

Veratridine has been known to cause a prolonged depolarization of membranes of squid and crayfish giant axons (Ohta *et al.*, 1973). If this is the case in the adrenal chromaffin cells, veratridine may cause a sustained catecholamine output resulting from the facilitation of the voltage-dependent Ca^{2+} influx. However, maintained K^+-depolarization was found first to activate and subsequently to inactivate voltage-dependent Ca^{2+} channels in squid axons (Baker, Meves & Ridgway, 1973) and the bovine adrenal chromaffin cells (Baker & Rink, 1975). Thus, it is of interest to examine whether or not prolonged exposure to veratridine causes a sustained increase in the catecholamine output.

Adrenal glands were perfused for 1 h with Locke solution containing veratridine (100 μM). In order to check the responsiveness of the chromaffin cells, they were also perfused with Locke solution containing excess K^+ at the beginning and the end of the experiments. The catecholamine output induced by veratri-

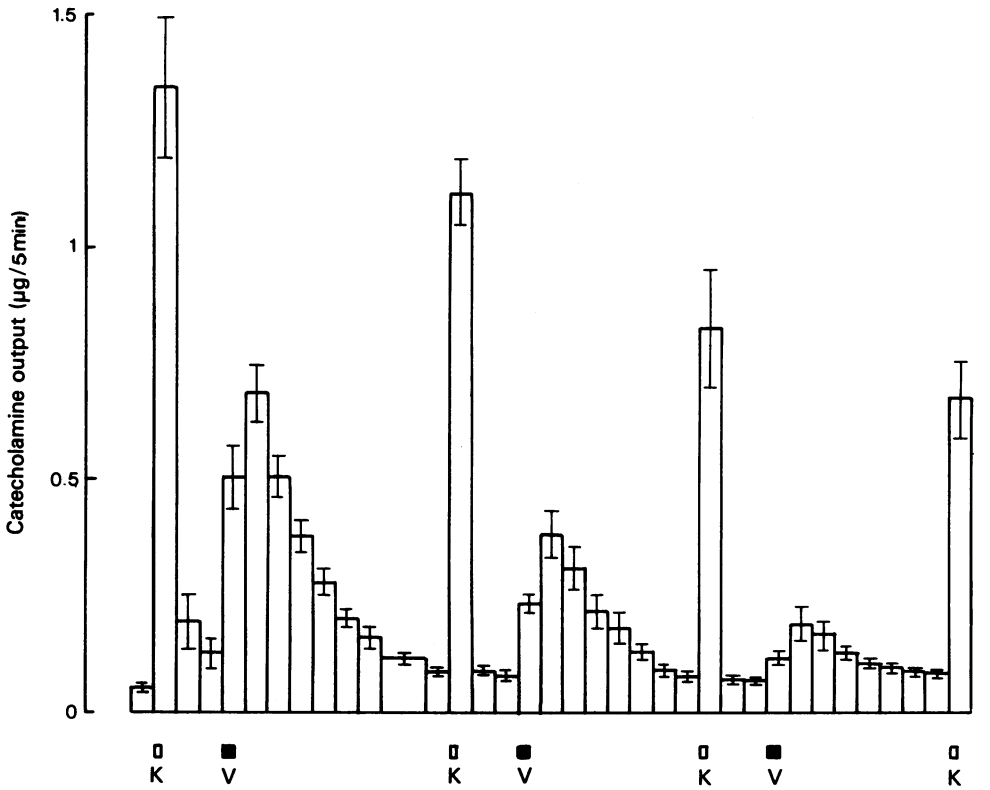

Figure 2 Catecholamine secretion induced by alternating perfusions with Locke solution containing an excess of potassium or veratridine. (□) Infusion of excess K^+ (56 mM, K) for 2 min, (■) infusion of veratridine (100 μM, V) for 3 minutes. Columns: mean (n = 7) output of catecholamines (μg/5 min), Vertical lines show s.e. mean. Wide column: 10 min collection period.

dine developed gradually, reaching its maximum within 5 to 10 min and then declined exponentially in spite of the presence of veratridine (Figure 3). However, an excess of K^+, infused after the termination of the veratridine-induced responses, was still effective in stimulating the catecholamine output, although the response was somewhat smaller. Similar results were obtained in another two experiments. Thus the decrease in catecholamine secretion during a prolonged exposure to veratridine is not due to the exhaustion of adrenal stores of releasable catecholamines.

Calcium dependency of the effect of veratridine on adrenal catecholamine secretion

As reported previously, veratridine failed to elicit catecholamine secretion in the absence of extracellular Ca^{2+} (Ito *et al.*, 1978). In the present experiments, the Ca^{2+}-dependency of the veratridine-induced response was confirmed by changing the extracellular concentration of Ca^{2+}. Figure 1 shows that the effect of veratridine was maintained even after its withdrawal from the perfusion medium. Thus the following experiments were carried out. Adrenal glands were perfused for 20 min with a Ca^{2+}-free solution containing veratridine. Subsequently the adrenal glands were perfused for 10 min with a veratridine-free solution containing Ca^{2+} in concentrations ranging from 0.55 to 8.8 mM.

A typical experiment is shown in Figure 4a. There was no increase in the catecholamine output during exposure to veratridine in the absence of Ca^{2+}. However, perfusion with Ca^{2+}-containing solutions enhanced the catecholamine output progressively with increasing concentration of Ca^{2+} reaching a maximum at 2.2 mM. An increase of the Ca^{2+} concentration to 4.4 mM did not cause a further increase in the catecholamine output. These results are not necessarily due to the fact that Ca^{2+} 4.4 mM is equipotent to 2.2 mM, because the stores of releasable catecholamines in the adrenal glands may have been exhausted. In addition, the effect of veratridine could have ceased at the time when 4.4 mM Ca^{2+} was infused. In order to rule out these possibilities, experi-

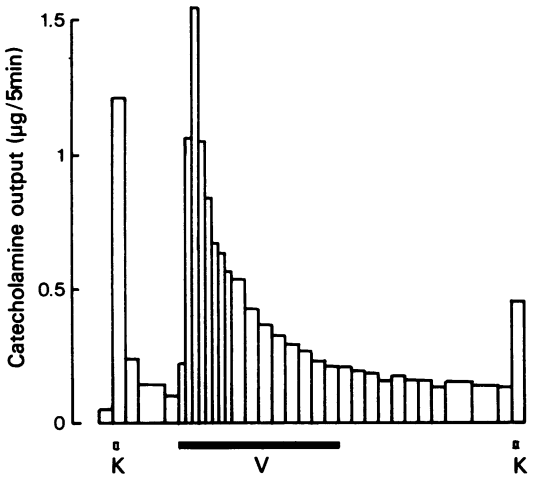

Figure 3 Catecholmaine output induced by prolonged infusion of veratridine. Veratridine (100 μM) was infused for 1 h (horizontal black bar, V). Excess K^+ (56 mM) was infused for 2 min (□, K) at the beginning and at the end of the experiment. During the 20 min period after the start of the veratridine infusion, adrenal effluent was collected for 2.5 min (narrow columns). Wide columns indicate 10 min collection periods. Similar results were obtained in two other experiments.

ments were carried out in which 4.4 mM or 8.8 mM Ca^{2+} solutions were infused immediately after the infusion of the 1.1 mM Ca^{2+} solution. The catecholamine amount released during the first 5 min of a perfusion with a given Ca^{2+} concentrations was expressed as a percentage of that obtained in the presence of 0.55 mM Ca^{2+} (=1) (see Figure 4b). The results show that the catecholamine output increased with increasing concentrations of Ca^{2+} ranging from 0.55 mM to 8.8 mM.

Sodium dependency of the catecholamine secretion induced by veratridine

According to Ohta *et al.* (1973), veratridine failed to depolarize the membranes of squid and crayfish giant axons in the absence of extracellular Na^+ ions. As reported previously, extracellular Na^+ ions were also found to be indispensable for the catecholamine release induced by veratridine (Ito *et al.*, 1978). A Na^+ channel was believed to discriminate poorly between Na^+ and Li^+ ions (Hille, 1970) and the action potential was little affected when extracellular Na^+ ions were replaced by Li^+ ions (Hodgkin & Katz, 1949; Huxley & Stämpfli, 1951). In the adrenal chromaffin cells, Li^+, unlike sucrose or choline, seems to substitute for Na^+ (Lastowecka & Trifaró, 1974). We have therefore investigated whether veratridine can evoke the catecholamine output in Na^+-free, Li^+-containing solutions.

When adrenal glands were perfused with Locke solution in which NaCe was replaced by LiCl, only a small increase in the catecholamine output was observed in the presence of extracellular Ca^{2+} (Figure 5a). This was in agreement with the results reported by Rink (1977). On the other hand, when NaCl was replaced by either choline chloride or sucrose, a significant response was obtained regardless of the presence (Figure 5b and Figure 8) or the absence (not shown) of extracellular Ca^{2+}. These results were consistent with the findings of Lastowecka & Trifaró (1974). Forty-five min after the replacement of Na^+ with Li^+ (Figure 5a) or choline (Figure 5b), the glands were perfused with veratridine. In both cases, veratridine failed to increase catecholamine output. Failure of veratridine to increase the catecholamine output does not seem to be due to the disturbance of the secretory apparatus caused by the long absence of Na^+, because subsequent introduction of Na^+ ions caused a large and sustained output of catecholamines (Figure 5a and b).

Inhibitory effects of tetrodotoxin and excess magnesium on the veratridine-induced release of catecholamines

Tetrodotoxin TTX has been reported to inhibit veratridine-induced responses, such as the depolarization of squid and crayfish giant axons (Ohta *et al.*, 1973) and rat brain synaptosomes (Blaustein & Goldring, 1975), the release of noradrenaline from peripheral adrenergic neurones (Thoa *et al.*, 1975) and of insulin from the pancreatic β-cells (Donatsch *et al.*, 1977), and Ca^{2+} uptake by rat brain synaptosomes (Blaustein, 1975).

As described above, adrenal glands were alternately perfused with veratridine for 3 min and excess K^+ (56 mM) for 2 min. TTX (0.1 μM or 1 μM) was added to the media 5 min before and during the second infusion of veratridine or excess K^+. Typical responses are illustrated in Figure 6a. Similar results were obtained in four other experiments. TTX was effective in inhibiting the catecholamine output induced by veratridine as reported previously (Ito *et al.*, 1978). TTX in concentrations of 0.1 μM or 1 μM was ineffective in inhibiting the response induced by excess K^+.

Magnesium It is well-known that excess Mg^{2+} inhibits the release of catecholamines from adrenal chromaffin cells induced by excess K^+ or by ACh (Douglas & Rubin, 1961; 1963). Therefore, the effect of excess Mg^{2+} (20 mM) on the release of catecholamines induced by veratridine was examined in an experiment similar to that with TTX. A typical result is shown in Figure 6b. Similar results were obtained

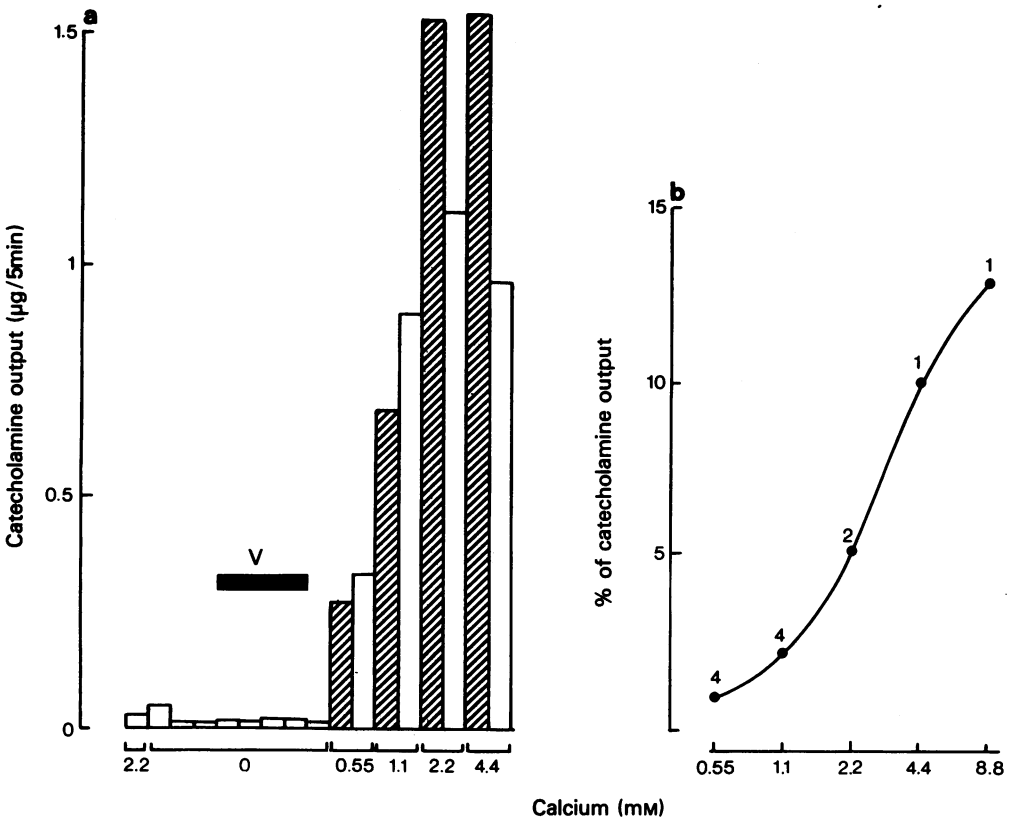

Figure 4 Effect of calcium on the stimulation by veratridine of the catecholamine secretion by the perfused guinea-pig adrenal gland: (a and b) adrenal perfused with modified Locke solution containing Tris amino-methane buffer and $MgCl_2$ (4 mM). In (a) veratridine (100 μM) was present for 20 min (black bar, V); (a) numbers below columns indicate Ca^{2+} concentrations present in perfusate. Each column represents the amount of catecholamines (μg) released in 5 minutes. Hatched columns: first 5 min after perfusion with the next high Ca^{2+}-concentration. (b) Catecholamines released from the perfused adrenal gland starting 5 min after a 20 min exposure to veratridine (100 μM). Abscissa scale: Ca^{2+}-concentrations in perfusate (logarithmic scale). Ordinate scale: catecholamines released as a percentage of the amount released in the presence of 0.55 mM Ca^{2+} (=1). The results with 4.4 or 8.8 mM Ca^{2+} were obtained on preparations that had previously only been perfused with 1.1 mM Ca^{2+} in order to avoid exhaustion of medullary catecholamine stores.

in two other experiments. Excess Mg^{2+} completely blocked the catecholamine output evoked by veratridine and reduced that induced by excess K^+.

Potentiation of the veratridine-induced catecholamine release by ouabain

Ouabain is known to cause an increase in the transmitter release from motor nerve endings (Elmqvist & Feldman, 1965a, b; Birks & Cohen, 1968a, b; Baker & Crawford, 1975) and from peripheral adrenergic neurones (Nakazato, Ohga & Onoda, 1978). It has been reported that ouabain caused an increase in the spontaneous release of catecholamine and in the potentiation of the output of catecholamines evoked by carbachol from the perfused bovine adrenal glands (Banks, 1967). Most of these authors suggested that the changes in the distribution of Na^+ and K^+ across the membranes, resulting from the inhibition of the Na^+ pump, may play a critical role in the release induced by ouabain. If so, ouabain should be effective in enhancing the veratridine-induced response, because more intracellular Na^+ ions are expected to be accumulated by exposure to veratridine during the inhibition of the Na^+ pump.

In seven experiments, the adrenal glands were perfused with veratridine (100 μM or 50 μM) for 3 min in the absence of ouabain. After the catecholamine output had returned to resting values, ouabain (10 μM) was added to the perfusion media starting 20 min

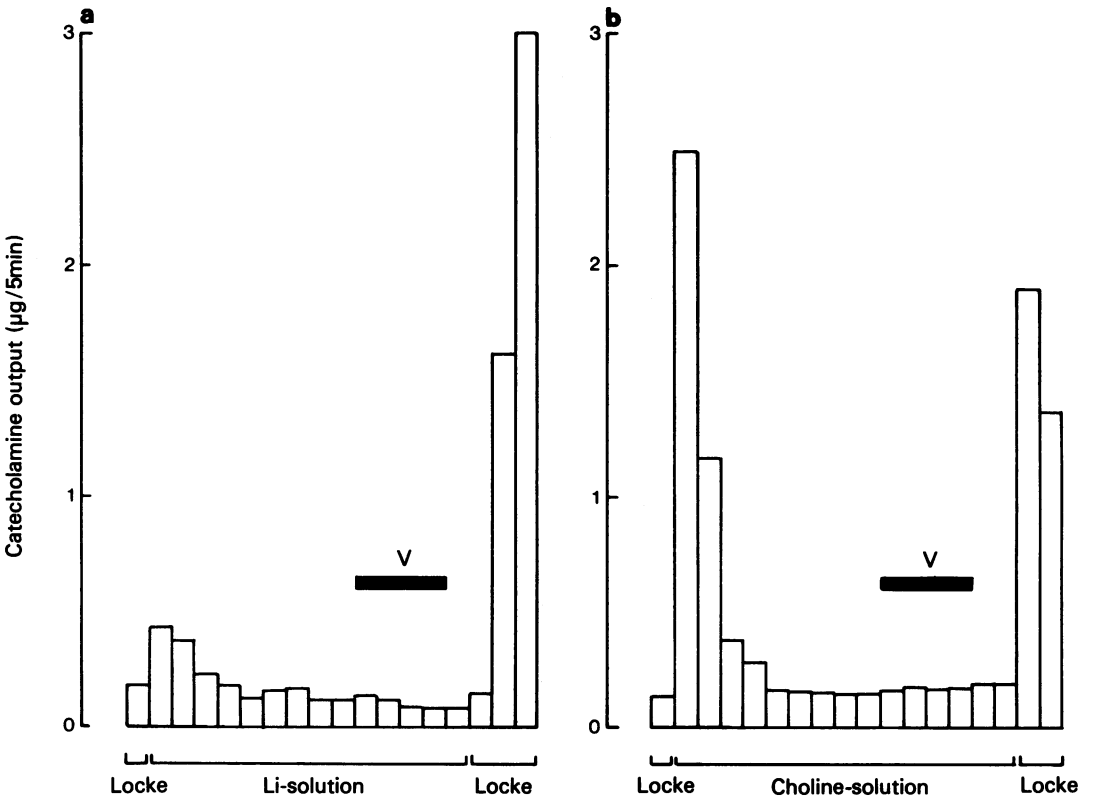

Figure 5 The effect of veratridine on adrenal catecholamine secretion in a Na^+-free medium. Adrenal glands were perfused with solutions containing Tris amino-methane buffer (2.5 mM). Forty-five min after the replacement of Na^+ with Li^+ (a) or choline (b), veratridine (100 µM, V) was added for 20 min (black horizontal bar). Solutions used for each period are indicated below histogram.

before the second application of veratridine and lasting to the end of the experiments. Under these conditions, ouabain caused an increase in the resting catecholamine output and significantly potentiated and prolonged the effect of veratridine (Figure 7).

Effect of veratridine on adrenal catecholamine secretion in a Na^+-free solution containing a high concentration of calcium

It was found that Ca^{2+} could enter the squid giant axon through Na^+ channels (Baker, Hodgkin & Ridgway, 1971). Furthermore, according to Baker (1972), Ca^{2+} entry through Na^+ channels still occurs even when Na^+ ions are moving out of the axon, for instance in Na^+-free water. Quite recently, veratridine-mediated insulin release obtained in the absence of extracellular Na^+ led Donatsch *et al.* (1977) to suggest that Ca^{2+} could enter the pancreatic β-cells through Na^+ channels. Our experiments have shown that veratridine failed to cause an increase in the catecholamine output in Na^+-free solutions containing Ca^{2+} (2.2 mM). Further experiments were carried out in which the effect of veratridine was studied in Na^+-free solutions containing a high concentration of Ca^{2+} (8.8 mM). The results are shown in Figure 8. When the perfusion medium was changed from Locke solution to a Na^+-free solution based on sucrose, a substantial catecholamine output occurred in confirmation of the observations by Lastowecka & Trifaró (1974) and also shown in a previous section. When catecholamine secretion was back to the resting level, the concentration of Ca^{2+} in the perfusion fluid was increased to 8.8 mM. Even under these conditions, veratridine failed to cause a statistically significant increase in the catecholamine output. Reintroduction of Na^+ into the perfusion medium caused a significant increase in the catecholamine output.

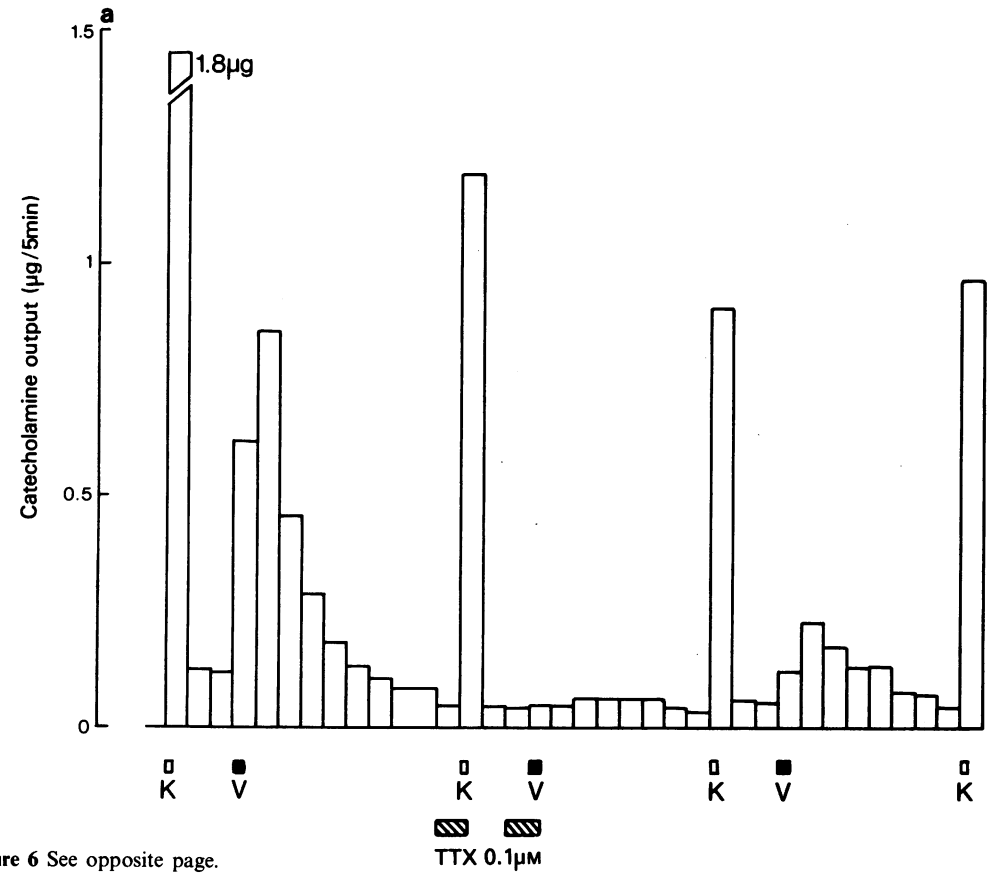

Figure 6 See opposite page.

Discussion

Our results indicate that Na^+ channels exist in adrenal chromaffin cell membranes and that veratridine directly activates these channels to produce the release of catecholamines. This release is dependent on extracellular Na^+ and Ca^{2+} ions. How does the effect of veratridine compare with the effect of excess K^+?

Voltage-dependent Ca^{2+} influx

Just as Ca^{2+} ions play a critical role in the effect of excess K^+ on the catecholamine output from perfused adrenal glands (Douglas & Rubin, 1963), the increase in catecholamine output induced by veratridine was dependent on the concentration of extracellular Ca^{2+} ions. Therefore, Ca^{2+} influx across chromaffin cell membranes seems to be involved in the effect of veratridine on these cells, as it is in the effect of excess K^+ ions.

The removal of extracellular Na^+ and the addition of TTX were also found to be effective in inhibiting veratridine-induced catecholamine release. Ohta *et al.* (1973) reported that, in the squid giant axons, veratridine caused a depolarization resulting from a selective increase in the resting Na^+ permeability and that the effect was inhibited by the addition of TTX and the removal of extracellular Na^+ ions. Our results, therefore, suggest that an increase in the Na^+ permeability of chromaffin cell membranes also plays a critical role in the release of catecholamine induced by veratridine. On the other hand, the catecholamine output induced by excess K^+ was not inhibited either by reduction of extracellular Na^+ concentration (Rink, 1977) or addition of TTX. Using synaptosomes isolated from rat brain, Blaustein (1975) found that Ca^{2+} uptake in response to excess K^+ was almost unaffected by TTX. Increasing extracellular concentrations of KCl caused a stable depolarization of chromaffin cell membranes resulting from changes in the K^+ equilibrium potential (Baker & Rink, 1975). Thus, unlike the effect of veratridine, the response induced by excess K^+ may not be related primarily to an increase in Na^+ permeability.

Excess Mg^{2+} inhibited catecholamine output in-

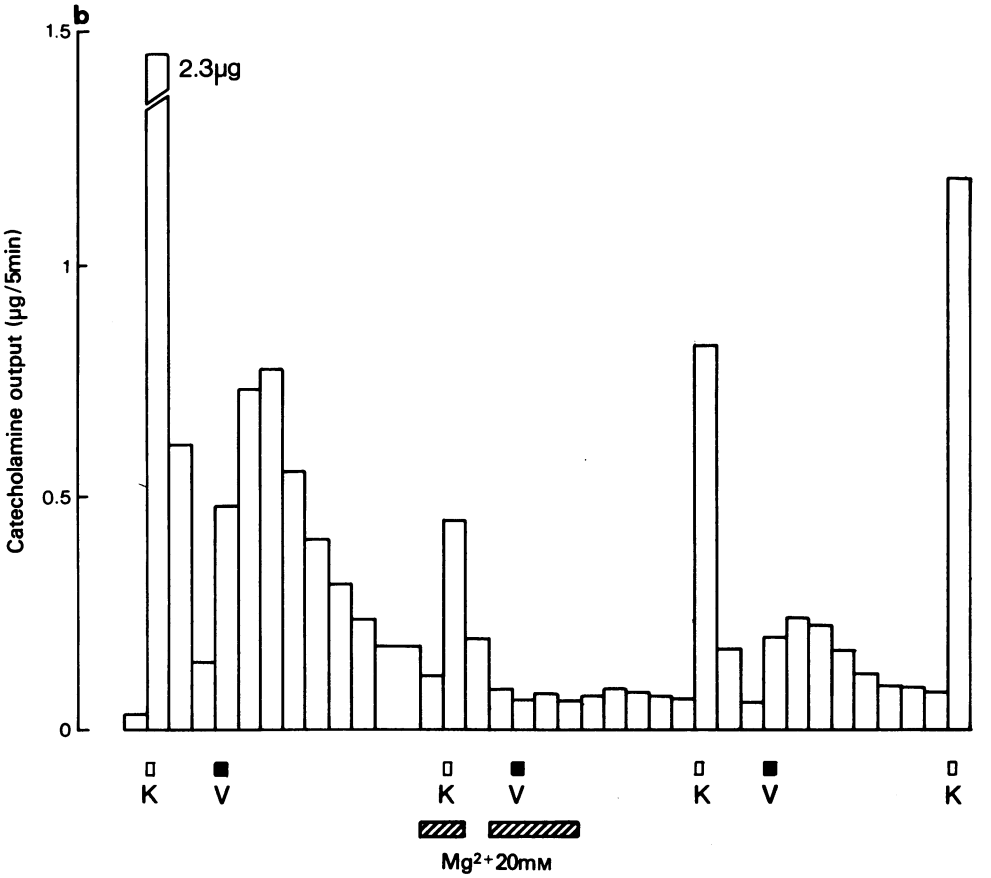

Figure 6 The inhibitory effects of tetrodotoxin (TTX) (a) and excess magnesium (b) on the responses induced by veratridine and excess potassium. Adrenal glands were perfused with Locke solution. Open (□) and filled (■) squares indicate the periods of the application of excess K^+ (56 mM, K) for 2 min and veratridine (100 μM, V) for 3 minutes. Horizontal hatched bars indicate the period of the presence of TTX (0.1 μM) in (a) and the presence of Mg^{2+} (20 mM) in (b).

duced by veratridine and by excess K^+. The effect of excess Mg^{2+} was much more pronounced towards the response induced by veratridine than that evoked by excess K^+. It is well known that Mg^{2+} ions as well as Ca^{2+} ions stabilize the excitability of nerve membranes by changing the permeabilities of monovalent cations (Frankenhaeuser & Hodgkin, 1957). This phenomenon was explained by the ability of divalent cations to screen the electrostatic effect of the fixed negative surface charges near Na^+ channels (Hille, Woodhull & Shapiro, 1975). Therefore, it seems likely that excess Mg^{2+} inhibits the effect of veratridine not only by competing with Ca^{2+} in the cell membranes (Douglas & Rubin, 1963) and/or inside the cell (Lastowecka & Trifaró, 1974; Aguirre *et al.*, 1977), but also by decreasing Na^+ permeability.

The increase in Na^+ permeability induced by veratridine is expected to cause depolarization of adrenal chromaffin cells, since in cultured adrenal chromaffin cells, increase in Na^+ permeability caused depolarization (Douglas, Kanno & Sampson, 1967b; Biales *et al.*, 1976; Brandt *et al.*, 1976). It was reported that K^+-induced depolarization first activated and subsequently inactivated voltage-dependent Ca^{2+} channels in squid axons (Baker *et al.*, 1973) and in adrenal chromaffin cells (Baker & Rink, 1975). In the present experiments, the catecholamine output elicited by veratridine gradually declined, even if veratridine was still present in the perfusion solution. Since veratridine was reported to cause sustained but slowly reversible depolarization of squid and crayfish giant axons (Ohta *et al.*, 1973), it is presumed that veratridine-

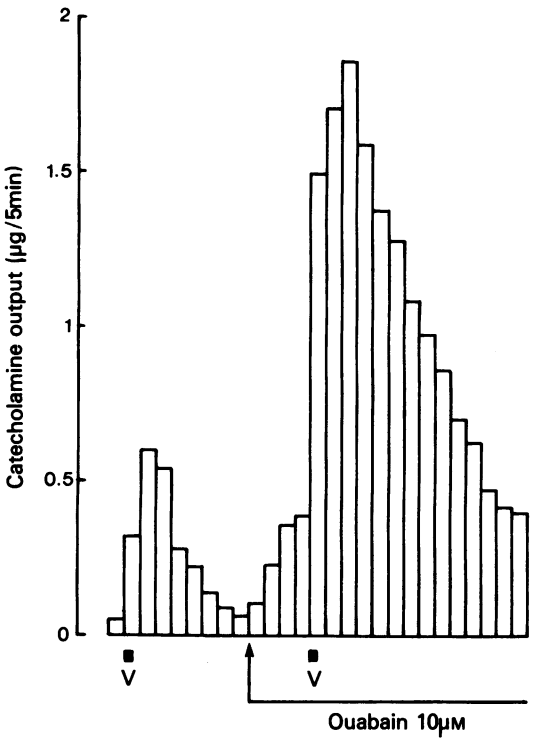

Figure 7 Potentiating effect of ouabain on the veratridine-induced catecholamine secretion from the perfused guinea-pig adrenal gland: (■) 3 min periods during which veratridine (50 μM, V) was present in the perfusion medium. Ouabain was present for the period indicated by a horizontal bar starting at the arrow.

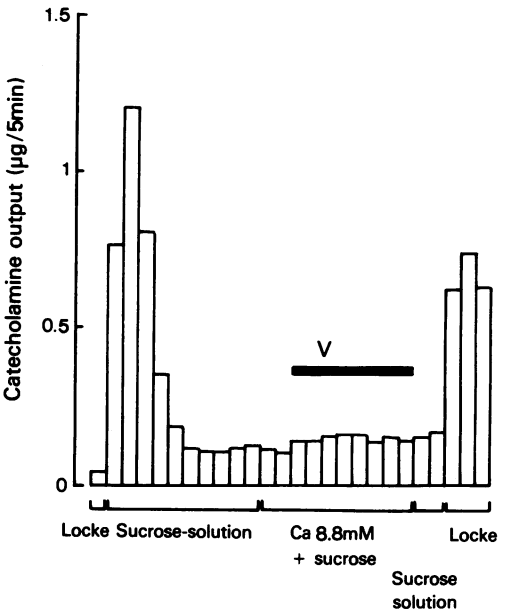

Figure 8 The effect of veratridine on adrenal catecholamine secretion in the presence of a high concentration of calcium ions (8.8 mM) in a Na^+-free medium. Na^+ was replaced by isotonic sucrose (sucrose-solution) containing Tris amino-methane buffer. Perfusion solutions and the concentration of Ca^{2+} are indicated below the histogram. Veratridine (100 μM, V) was present for the period indicated by the thick horizontal bar.

induced depolarization also causes an activation followed by inactivation of voltage-dependent Ca^{2+} channels in the chromaffin cell membranes.

Na^+-dependent Ca^{2+} influx

The failure of veratridine to cause an increase in the catecholamine output in the absence of Na^+ ions was not due to the lack of the drug binding to the adrenal chromaffin cell membranes, because the reintroduction of Na^+ ions after withdrawal of veratridine caused a substantial increase in catecholamine output. This restoration of the catecholamine output may be further evidence for the involvement of Na^+ ions in the veratridine-induced response.

Li^+ ions could not substitute for Na^+ ions to produce catecholamine output in response to veratridine. There are at least three possible interpretations of this. First, veratridine may have failed to cause depolarization in the presence of Li^+ ions because Li^+ could not pass through the Na^+ channels of chromaffin cell membranes. Secondly, Li^+ itself may have caused the depolarization of chromaffin cell membranes so that veratridine was no longer effective in increasing the Li^+ permeability. Thirdly, Li^+ could pass through Na^+ channels, but may not have behaved like Na^+ inside the cell, i.e. Li^+ failed to increase the permeability to Ca^{2+}.

It was reported that Li^+ ions could pass through Na^+ channels in neurones (Chandler & Meves, 1965), and thus Li^+ ions as well as Na^+ ions could maintain nerve activity (Hodgkin & Katz, 1949; Huxley & Stämpfli, 1951). In agreement with the finding of Rink (1977) but not with those of Lastowecka & Trifaró (1974), exposure of adrenal chromaffin cells to Na^+-free, Li^+-containing solution caused a small increase in the catecholamine output, if extracellular Ca^{2+} was present. However, from experiments with the fluorescent dye, diS-C_3-(5), Rink (1977) also reported that Li^+ solution had little effect on the membrane potential. These observations do not support the first and second possibility.

In squid giant axons, it is known that anything reducing the electrochemical gradient for Na^+ ions, for instance a rise in intracellular Na^+ or a fall in

extracellular Na^+, will tend to reduce the Na^+-dependent Ca^{2+} efflux and increase Ca^{2+} uptake in exchange for Na^+ loss (Baker, 1972). This mechanism was adopted by Nakazato, Onoda & Ohga (1977) and Nakazato *et al.* (1978) to interpret the increase in the noradrenaline output from peripheral adrenergic neurones induced by Na^+ deprivation and ouabain. Quite recently, it was demonstrated that Na^+-dependent Ca^{2+} efflux and Ca^{2+}-dependent Ca^{2+} efflux mechanisms were acting on the adrenal chromaffin cell membranes (Aguirre *et al.*, 1977; Rink, 1977) as well as the squid axon membranes (Baker, 1976). Therefore, Na^+-dependent Ca^{2+} influx, a reversed Na^+-dependent Ca^{2+} efflux, could also be possible in adrenal chromaffin cell membranes as reported in squid axon membranes (Blaustein, 1976). If so, Na^+ ions which entered the chromaffin cell on exposure to veratridine may be responsible for the entry of extracellular Ca^{2+} ions by exchange. In contrast, Li^+ ions may not be effective in transporting Ca^{2+} by such an exchange mechanism. This explanation is supported by the significant potentiating effect of ouabain on the veratridine-induced response. Because Na^+ ions that entered the cell in response to veratridine were considered to be liberated continuously from the cell by activating a Na^+ pump, the inhibition of the Na^+ pump by ouabain should result in further accumulation of intracellular Na^+ ions. According to Blaustein (1975), Ca^{2+} uptake induced by veratridine-like depolarizing agents such as excess K^+ was decreased by the addition of ouabain in the rat brain synaptosomes. This is incompatible with our result. The reason for this discrepancy may be that Blaustein (1975) exposed the synaptosomes to veratridine only for 30 to 60 s in order to minimize the changes in intracellular Na^+ which activates the Na^+-dependent Ca^{2+} influx system.

Ca^{2+} influx passing through Na^+ channels

It was found that Ca^{2+} could enter the squid giant axon through Na^+ channels (Baker *et al.*, 1971). Quite recently, Donatsch *et al.* (1977) found that veratridine increased insulin release in the absence of Na^+ and suggested that Ca^{2+} could enter the pancreatic β-cells through Na^+ channels. In our experiments, veratridine failed to induce catecholamine output in Na^+-free sucrose solution containing a high concentration of Ca^{2+} (8.8 mM). This result suggests that Ca^{2+} cannot enter the adrenal chromaffin cells through Na^+ channels or that, unlike the pancreatic β-cells, Ca^{2+} passing through Na^+ channels is not involved in the release of catecholamines.

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(Received July 21, 1978.)