Effects of Veratridine on Ca Fluxes and the Release of Oxytocin and Vasopressin from the Isolated Rat Neurohypophysis

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ABSTRACT Uptake of radioactive calcium, ^{45}Ca efflux, and hormone release from the isolated rat neurohypophysis were monitored in vitro after the addition of veratridine to the incubation medium. Veratridine dramatically increased hormone release, but the release rate was not sustained and had declined by about 90% after 2 h. Removal of external Na^+ prevented hormone release as did addition to the incubation medium of tetrodotoxin or the calcium antagonists D600 and Mn^{2+} ions. Veratridine increased ^{45}Ca uptake into the isolated neurohypophysis and the increase could be prevented by addition of tetrodotoxin or D600 to the medium. Efflux of ^{45}Ca was not changed by addition of veratridine. The results underline the importance of both Na^+ and Ca^{+2} channels in the regulation of secretion of neurosecretory products.

INTRODUCTION

There is increasing evidence (see Douglas, 1974) that the release of neurohormones or neurotransmitters is dependent upon the cytoplasmic calcium ion concentration, probably at the internal face of the secretory membrane. An increase in the cytoplasmic calcium concentration is sufficient to trigger release. Evidence for this is that an increase in the quantal release of neurotransmitters at the squid stellate ganglion can be observed when calcium ions are injected presynaptically (Miledi, 1973). Furthermore, release of calcium from intracellular sources can also promote release of neurotransmitters or neurohormones (Alnaes and Rahamimoff, 1975; Nordmann and Currel, 1975).

To characterize the role of Na channel in the release process at the neurohypophysial nerve terminals, we investigated the effects of veratridine on hormone release and calcium movements in the neurohypophysis. Veratridine is known to depolarize excitable cells (Straub, 1956; Ulbricht, 1969) by holding the sodium channels in an open state (Ohta et al., 1973).

Our results suggest that Na channels are present in the terminals of neurosecretory nerves. Under normal conditions these Na channels are probably responsible for depolarization of the plasmalemma. Such a depolarization leads in turn to activation of "late" calcium channels (Baker et al., 1971) and promotes hormone release.

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METHODS

The experiments were carried out on isolated neural lobes of male rats (Porton, Wistar) weighing 200–250 g. The tissue was dissected out after decapitation of the animals and incubated for 20 min in normal Locke's solution and then transferred either to a medium containing 100 mM Na^+ or a sodium-free medium which was changed every 15 min.

Composition of Medium

Normal Locke's solution: NaCl, 150 mM; $CaCl_2$, 2.2 mM; $MgCl_2$, 1.0 mM; $KHCO_3$, 5.6 mM; glucose, 10 mM maintained at 37°C and gassed with 5% CO_2 in O_2. When NaCl was reduced the tonicity was maintained with choline chloride.

^{45}Ca Uptake Experiments

Radioactive calcium (10 μCi ml^{-1}) was added to the medium for the relevant 15-min period. After the incubation the glands were immersed for 1 h in a sodium-free medium which was changed every 10 min to remove ^{45}Ca from the extracellular spaces. Sodium-free medium was used because, in such a medium, Ca efflux from the neurohypophysis is reduced (Dreifuss and Nordmann, 1974). The glands were then blotted dry and digested overnight in soluene (Packard Inst. Co., Downers Grove, Ill.), and the ^{45}Ca was counted after adding 10 ml of scintillation fluid made up as follows: 2-methoxyethanol, 250 ml; ethoxyethanol, 500 ml; toluene, 750 ml; butyl PBD, 9 g.

^{45}Ca Efflux Experiments

Groups of four glands were loaded for 60 min in a solution containing 33 μCi ml^{-1} ^{45}Ca. They were then transferred to a Na-free, or Na and Ca-free medium which was changed every 10 min for 2 h. The efflux of ^{45}Ca, calculated from the radioactivity in each sample, was expressed as a fractional rate constant, given by the fractional efflux (min^{-1}) = $(\Delta x)/(\Delta t\, x_t)$, where Δx represents counts/min ^{45}Ca released in the time interval Δt, and x_t the tissue ^{45}Ca content at the midpoint of interval Δt.

Hormone Assay

The released neurohypophysial hormones were estimated by the rat milk ejection method (Bisset et al., 1967) using Pitocin (Parke, Davis & Co., Detroit, Mich.) as a reference standard. It should be pointed out that this assay measures both oxytocin and vasopressin at a ratio of about 7:1.

In few experiments, oxytocin and vasopressin and total neurophysin (I + II) were measured using radioimmunoassays (Legros et al., 1971).

Chemicals

^{45}Ca was supplied by the Radiochemical Centre, Amersham, England. Veratridine was obtained from Serva, Heidelberg, West Germany. D600 (racemic mixture) was a gift from Knoll AG., Ludwigshafen, West Germany. All chemicals were of analar grade.

RESULTS

The Effect of Veratridine on Neurohormone Release

Neurohypophyses incubated in normal saline containing 7.5×10^{-5}M veratridine released 37.85 ± 4.60 mU (mean ± SE) hormone (Table I). This value was obtained using the bioassay (see Methods). A similar increase was observed when a specific radioimmunoassay was used to measure oxytocin, vasopressin, and neurophysin output (Table II). Release at this rate was not maintained

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during long exposure to the alkaloid. Fig. 1 shows that the decay of hormone release during prolonged exposure to veratridine can be described by a single exponential having a rate constant of 0.027 and a half-time of 26 min. The decline in hormone release has been shown not to be due to some nonspecific

TABLE I

EFFECTS OF VERATRIDINE ON HORMONE RELEASE

Conditions*	Hormone released‡	n§	ΔH‖
	mU/ml per 15 min		*mU*
100 Na	2.0±0.2	10	–
100 Na+veratridine	37.8±4.6	14	35.8
0 Na	3.5±0.6	4	–
0 Na+veratridine	4.3±0.1	4	0.8
100 Na+TTX	2.0±0.2	3	–
100 Na+TTX+veratridine	1.6±0	3	−0.4
100 Na+D600	1.9±0.1	4	–
100 Na+D600+veratridine	2.0±0.2	4	0.1
100 Na+Mn	2.3±0.3	4	–
100 Na+Mn+veratridine	5.7±1.7	4	3.4

* Veratridine, 7.5×10^{-2} mM; TTX, 10^{-6} g/ml; D600, 10^{-1} mM; Mn^{2+}, 5.0 mM.
‡ Mean ± SE.
§ Number of experiments.
‖ Increase in hormone release due to veratridine.

TABLE II

EFFECTS OF VERATRIDINE ON OXYTOCIN, VASOPRESSIN, AND TOTAL NEUROPHYSIN RELEASE

Conditions*	Oxytocin‡	Vasopressin‡	Neurophysin‡	n§
		ng/ml per 15 min‖		
100 Na	4.4±0.9	3.5±1.0	18.0 ± 2.1	3
100 Na +veratridine	85.4±9.6	52.8±7.5	98.0 ± 14.0	3
100 Na +D600	3.9	3.0	10.3	¶
	2.0	2.0	–	
100 Na +D600	3.0	3.0	12.5	¶
+veratridine	2.0	2.0	17.0	

* Veratridine, 7.5×10^{-2} mM; D600, 10^{-1} mM.
‡ Measured by radioimmunoassays.
§ Number of experiments.
‖ Mean ± SE.
¶ Two experiments. Both values are given.

decay of the gland during prolonged incubation (Nordmann, 1976; Dyball and Nordmann, 1977).

Removal of external sodium almost completely blocked the response to veratridine (Table I). Table I shows that in the presence of the Na-channel

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blocking agent tetrodotoxin (TTX), 10^{-6} g/ml, the response to veratridine was totally abolished. Furthermore, in the presence of Mn^{2+} ions (Table I), at a concentration known to abolish completely the release evoked by 56 mM KCl (Dreifuss et al., 1973), the response to veratridine was reduced by 83%. At higher veratridine concentration (15×10^{-5} M), Mn^{2+} ions reduced the hormone release only by 54%. Tables I and II also shows that D600 (10^{-4}M), which entirely abolished potassium-induced hormone release (Dreifuss et al., 1973), totally inhibited the secretory effect of veratridine. A blocking effect of D600 + isomer (see Rasmussen and Goodman, 1977) on the "fast channel" is unlikely inasmuch as it is observed only at higher concentrations.

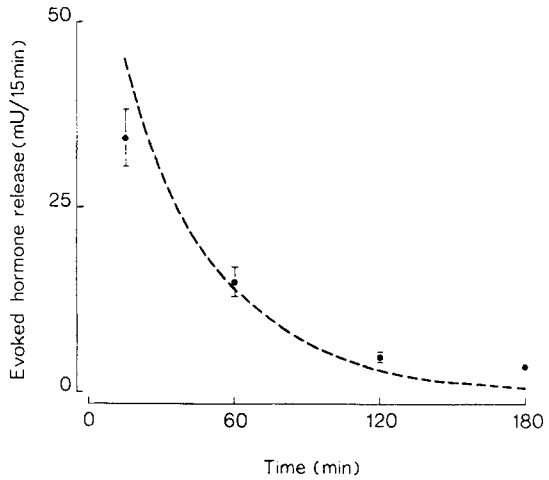

FIGURE 1. Effects of continuous exposure to veratridine on hormone release. Abscissa: time in minutes. Ordinate: milliunits of hormone released per 15 min evoked by veratridine (7.5×10^{-2} mM); mean ± SE, $5 \leq n \leq 14$. To calculate the evoked hormone release one subtracts the basal resting value from the value observed during the period of stimulation. From the data obtained a curve was reconstructed according to

$$H_t = 68\, e^{-0.027\, t},$$

where H_t corresponds to the hormone release at time t after the addition of veratridine ($r^2 = 0.96$, $t_{\frac{1}{2}} = 26$ min).

In some experiments oxytocin, vasopressin, and total neurophysin found in the medium were measured using radioimmunoassays. The results are summarized in Table II.

The Effect of Veratridine on ^{45}Ca Movements

Table III shows that veratridine gave rise to an increased uptake of ^{45}Ca which was totally abolished in the absence of external sodium or in the presence of tetrodotoxin. Furthermore, D600, which blocked the ^{45}Ca-uptake evoked by 56 mM KCl (Dreifuss et al., 1973) also blocked the veratridine dependent calcium uptake (Table III).

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To find out if, in the posterior pituitary, intracellular sequestered calcium can be released during exposure to veratridine, we loaded neurohypophyses with ^{45}Ca and studied the effect of veratridine on the slow phase (Fig. 2) of the calcium efflux. The alkaloid did not change the calcium efflux significantly when the glands were washed in a Ca^{2+}-containing (Fig. 2) or Ca^{2+}-free solution. The mean rate constant during the 40 min preceeding the addition of veratridine was 0.0055 ± 0.0005 (mean ± SE) whereas it was 0.0051 ± 0.0004 during the 40 min of exposure to 7.5×10^{-5} M veratridine in a Ca-containing saline.

DISCUSSION

Release of hormone from neurosecretory nerve terminals is known to be calcium-dependent. Douglas and Poisner (1964 *a*) showed that depolarized neurohypophyses release neurohormones only in the presence of external calcium ions. They also showed that hormone release is associated with an uptake of extracellular calcium (Douglas and Poisner, 1964 *b*). Later work showed that the calcium uptake responsible for hormone release occurs through

TABLE III

EFFECTS OF VERATRIDINE ON ^{45}Ca UPTAKE

Conditions*	Uptake	n§	ΔU‖
	cpm/15 min/mg protein‡		*cpm*
100 Na	220±27	9	–
100 Na+veratridine	552±38	19	302
0 Na	246±35	5	–
0 Na+veratridine	139±10	5	−107
100 Na+TTX	230±20	5	–
100 Na+TTX+veratridine	201±10	5	−29
100 Na+D600	190±22	5	–
100 Na+D600+veratridine	183±12	5	−7

* Veratridine, 7.5×10^{-2} mM; TTX, 10^{-6} g/ml; D600, 10^{-1} mM; Mn^{2+}, 5.0 mM.
‡ Mean ± SE.
§ Number of experiments.
‖ Increase in ^{45}Ca uptake due to veratridine.

a calcium channel similar to the "late" calcium channel described in the squid axon (Baker et al., 1973) inasmuch as Mn^{2+}, Co^{2+}, and D600 were shown to block the potassium-induced calcium uptake and hormone release (Dreifuss et al., 1973).

Furthermore, potassium-induced depolarization of the neural lobe in Na-free media gives rise to the release of a large amount of vasopressin (Douglas and Poisner, 1964 *a*). This suggests that during depolarization of the membrane, calcium channels in the terminal are activated, resulting in a calcium influx and hormone release. The "late" calcium channel at the neurohypophysial endings is thought to be voltage- and time-dependent, for evoked calcium uptake is abolished when the membrane is repolarized to resting level and ^{45}Ca-uptake wanes when depolarization is maintained (Nordmann, 1976).

Our results suggest that neurosecretory nerve endings have TTX-sensitive Na channels as ^{45}Ca uptake and hormone release occur in the presence of

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veratridine. Furthermore, these effects are blocked in the presence of tetrodotoxin. Similar results have been reported in preparation of synaptosomes (Blaustein, 1975; Blaustein and Goldring, 1975). The veratridine-induced depolarization is likely to be due to the entry of sodium into the cell because no calcium uptake (Table III) nor hormone release (Table I) occurs in Na-free veratridine-containing solution. This is in contrast with the endocrine pancreas where veratridine can still produce a significant release of insulin in the absence of external sodium (Donatsch et al., 1977). These authors very reasonably suggested that the observed secretion might come from release of calcium from intracellular sources and also from the entry of some calcium ions through the TTX-sensitive Na-channel. In our preparation, this is unlikely for there is no increase of calcium efflux (Fig. 2) in the presence of the alkaloid.

The effect of veratridine on ^{45}Ca uptake and hormone release is mediated by

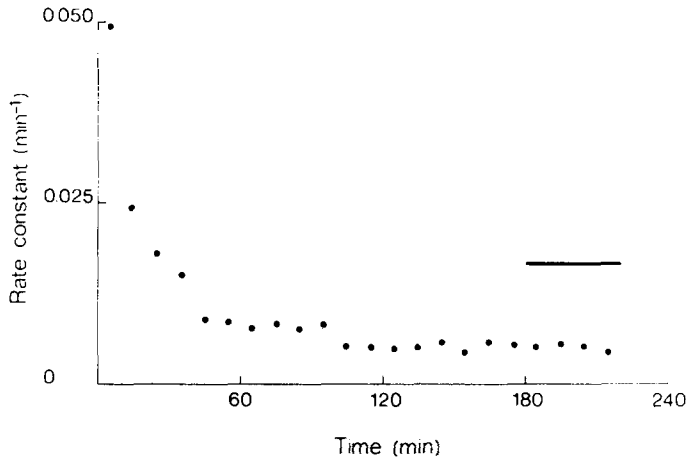

FIGURE 2. Effects of veratridine on the ^{45}Ca efflux from isolated neurohypophyses. Abscissa: time in minutes. Ordinate: rate constant in minutes^{-1}. Veratridine (7.5×10^{-2} mM) was present at the time indicated by the heavy bar. Each point represents the mean of four experiments.

the opening of the "late" calcium channel, for in the presence of veratridine, calcium uptake (Table III) and hormone release (Table I) are blocked by Ca^{2+} antagonist agents. It should, however, be pointed out that in the presence of 15×10^{-2} mM veratridine, Mn^{2+} ions did not completely block hormone release at a concentration which totally abolished the potassium-induced oxytocin secretion. These results are difficult to intepret. The small but significant veratridine-induced increase in hormone release in the presence of Mn^{2+} ions cannot be explained by the entry of calcium through the TTX-sensitive Na-channel for neither uptake nor release are observed in the absence of sodium (Table I). As already mentioned, mobilization of internal calcium is unlikely to be responsible. It thus appears that veratridine promotes the entry of calcium through the late calcium channels, but that some of the channels cannot be blocked by 5 mM Mn^{2+}.

The decreased hormone release during prolonged exposure to veratridine is

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probably not due to depletion of available hormone because the amount released represents only a small proportion of the total hormone store. Furthermore, although the amount of hormone release declines during prolonged depolarization, it has been shown that one can reactivate hormone release using a Ca ionophore (Nordmann, 1976), changes in the external sodium concentration (Müller et al., 1975; Nordmann, 1976) or veratridine (Dyball and Nordmann, 1977). It has thus been suggested that the inability of the gland to maintain a sustained release during prolonged stimulation is likely to be due to the inactivation of the late calcium channel observed during prolonged K^+ depolarization (Nordmann, 1976).

Our data suggest that the arrival of action potentials at the nerve terminals gives rise to a Na-dependent depolarization followed by the opening of the "late" calcium channel. The resulting increase in the ionized calcium concentration then promotes release of the neurohormones by exocytosis.

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