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MANGANESE IONS AND SYNAPTIC TRANSMISSION IN THE SUPERIOR CERVICAL GANGLION OF THE CAT

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1 In the perfused superior cervical ganglion of the cat manganese ions (2.7 – 4.6 mM) caused a block of synaptic transmission and reduced the output of acetylcholine.

2 Calcium ions (8.4-10.5 mM) relieved the synaptic block produced by manganese and partially restored the acetylcholine output.

3 The presence of manganese also reduced the sensitivity of ganglion cells to injected acetylcholine.

4 Both effects of manganese were completely reversible.

Introduction

Kostial & Juričić (1956) have shown that manganese ions block synaptic transmission in the superior cervical ganglion of the cat and that this block can be relieved by increasing the calcium ion concentration in the perfusion fluid. Since an increase in the calcium ion concentration is capable of restoring the output of acetylcholine from preganglionic nerve endings, the indication was that manganese ions reduced the output of transmitter as do magnesium ions (Hutter & Kostial, 1954).

In view of the role of calcium in excitation-secretion and excitation-contraction coupling, the action of manganese ions on transmitter release has recently received renewed attention (Kajimoto & Kirpekar, 1972; Kosterlitz & Waterfield, 1972; Meiri & Rahamimoff, 1972).

In the present experiments, direct evidence will be presented that manganese ions decrease the output of acetylcholine from preganglionic nerve endings. In addition it will be shown that manganese ions reduce the acetylcholine sensitivity of ganglion cells in common with some other divalent cations (Stanbury, 1948; Šlat & Kostial, 1959).

Methods

The experiments were performed on cats weighing 3-4 kg. Anaesthesia was induced with ether and maintained by an intravenous injection of chloralose (0.8 g/kg). The superior cervical ganglion was prepared for perfusion by the conventional method modified by Perry (1953). The preganglionic trunk was stimulated through platinum electrodes with supramaximal square voltage pulses of 1 ms duration. The contractions of the nictitating membrane were recorded with an isotonic lever writing on smoked paper and served to indicate the degree of ganglionic blockade. In some experiments acetylcholine (10-30 μg in 0.1 ml) was injected into the arterial cannula and the response of the nictitating membrane, recorded on the kymograph, was taken as an index of the sensitivity of the ganglion cells to acetylcholine.

When acetylcholine release from the ganglion was to be determined, physostigmine sulphate (10 μg/ml) was added to the perfusion fluid and the post-ganglionic trunk was tied. Generally 90% or more of the inflowing fluid was collected from the venous cannula in the first 1-2 h of the experiment but in all experiments care was taken to avoid accumulation of fluid around the ganglion, which remained *in situ* surrounded by liquid paraffin. After collection, the perfusate was acidified to pH 4-5 by addition of 0.1 N HCl and kept on ice until assay.

The ganglion was perfused with Locke solution of the following composition (mM): Na^+ 155.6; K^+ 5.6; Ca^{++} 2.1; Cl^- 163.6; HCO_3^- 1.8; and glucose (1 g/litre). The perfusion rate ranged from 0.7 to 1 ml/minute. Manganese and calcium were always added to Locke solution as anhydrous chlorides to give the desired concentrations as indicated in the text without altering the other components. On each change-over from one solution to another the cannula in the carotid artery was flushed out and the dead space

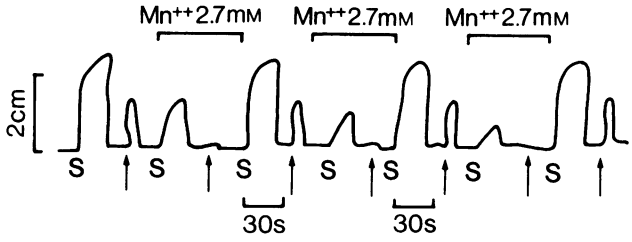

Fig. 1 Contractions of the cat nictitating membrane to stimulation of the preganglionic nerve at 2 Hz for 30 s (S) and to intra-arterial injection of acetylcholine (20 μg in 0.1 ml) at arrows during perfusion of the superior cervical ganglion with Locke solution or with Locke solution containing manganese chloride (2.7 mM). Addition of manganese caused partial block of the nictitating membrane contractions to preganglionic nerve stimulation and eventually abolished the response to injection of acetylcholine. All effects were completely reversible on subsequent perfusion with Locke solution.

remaining on the arterial side was thus reduced to less than 0.1 ml.

The acetylcholine was assayed on the blood pressure of eviscerated cats anaesthetized with chloralose and weighing 1-2 kg. The results were expressed in terms of the salt. The assay procedure was complicated by the fact that manganese itself produces a transient fall in blood pressure. Manganese chloride (2.7 mM) was added to all 1 ml samples for assay, i.e. to both standard (acetylcholine-containing) and test solutions.

Results

The effect of manganese on nictitating membrane contractions

In the experiment shown in Fig. 1, a ganglion was perfused alternately with Locke solution and with Locke solution containing manganese (2.7 mM). During each period the preganglionic nerve was stimulated at 2 Hz for 30 s and an intraarterial injection of acetylcholine (20 μg) was made. It can be seen that whenever manganese was present, the rate of rise of the contraction of the nictitating membrane to preganglionic stimulation was less and the amplitude reduced compared with that obtained during perfusion with Locke solution. The response to injected acetylcholine was almost completely suppressed in the presence of manganese and it also recovered whenever Locke solution was re-admitted.

In some experiments the response to injected acetylcholine was decreased by concentrations of manganese which had little effect on the response to nerve stimulation (Figure 2).

Figure 3 illustrates an experiment in which a ganglion was exposed to Locke solution containing manganese (4.6 mM). This had the effect of

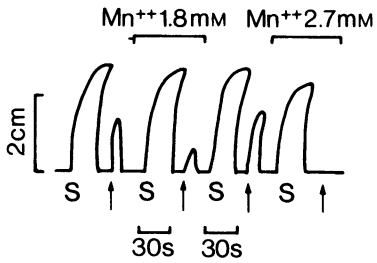

Fig. 2 Contractions of the cat nictitating membrane to stimulation of the preganglionic nerve at 2 Hz for 30 s (S) and to intra-arterial injections of acetylcholine (20 μg in 0.1 ml) at arrows during perfusion of the superior cervical ganglion with Locke solution or with Locke solution containing increasing concentrations of manganese (1.8 mM; 2.7 mM). The response to injected acetylcholine was decreased at concentrations of Mn which had little effect on the response to nerve stimulation.

causing a substantial decrease in the response to preganglionic nerve stimulation and a complete suppression of the response to injected acetylcholine. After a period in Locke solution the ganglion was exposed to a solution containing the same manganese concentration as before but this time the calcium concentration was raised to four times the normal value. The increase in the calcium content of the Locke solution led to the restoration of the response to preganglionic stimulation. In contrast, the response to injected acetylcholine remained completely suppressed.

The effect of manganese on acetylcholine output

In a superior cervical ganglion perfused with Locke solution the output of acetylcholine remains relatively constant if 5 min periods of pre-

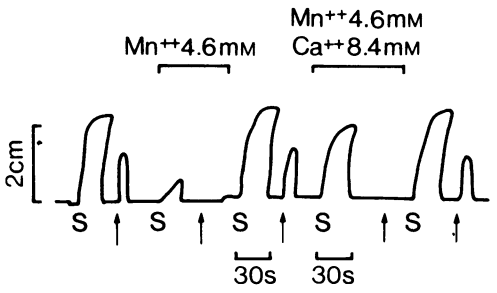

Fig. 3 Contractions of the cat nictitating membrane to stimulation of the preganglionic nerve at 2 Hz for 30 s (S) and to intra-arterial injection of acetylcholine (20 μg in 0.1 ml) at arrows during perfusion of the ganglion with Locke solution, Locke solution containing manganese (4.6 mM) and Locke solution containing manganese (4.6 mM) and increased calcium concentration (8.4 mM). By increasing the calcium concentration in presence of manganese the response of the membrane to stimulation was almost completely restored but the response to acetylcholine remained impaired.

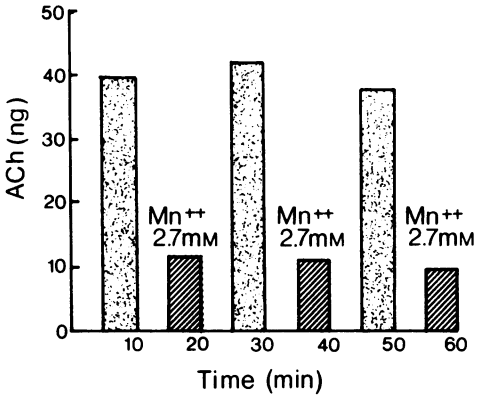

Fig. 4 Output of acetylcholine (ACh) from a ganglion perfused with Locke solution (physostigmine 10 μg/ml). Manganese (2.7 mM) was added to Locke solution in samples S_2, S_4, S_6 (Table 1a). The ganglion was perfused for 5 min before and 5 min during the stimulation period. Each column represents the acetylcholine released during 5 min of stimulation of the preganglionic nerve at 2 Hz followed by a 5 min resting period.

ganglionic stimulation at 2 Hz are followed by 5 min resting periods (Hutter & Kostial, 1954). Using this procedure the effect of addition of magnanese (2.7 mM) to Locke solution was tested in three ganglia. Figure 4 illustrates the result from one of these experiments, each column representing the amount of acetylcholine released over a 5 min period. The results of all three experiments are summarized in Table 1a which shows that manganese in the concentrations used decreased the output of acetylcholine to about 25% of its value in Locke solution.

Figure 5 illustrates the antagonistic effect of calcium ions on the reduction of acetylcholine release caused by manganese chloride (2.7 mM). In this experiment the addition of manganese to the perfusion fluid caused a reduction of the acetylcholine release to about 30% of the mean value in Locke solution. Subsequent addition of five times the normal amount of calcium to the perfusion fluid containing manganese (2.7 mM) restored the output of acetylcholine to about 75% of its control value. The results of two similar experiments are incorporated in Table 1b.

Table 1 The effect of manganese on acetylcholine release in the superior cervical ganglion of the cat

(a)			*(b)*		
Perfusion fluid	*Sample number*	*Release of ACh (ng)*	*Perfusion fluid*	*Sample number*	*Release of ACh (ng)*
Locke	S_1	44.5 ± 2.9	Locke	S_1	55.7 ± 2.8
Locke + Mn 2.7 mM	S_2	10.2 ± 0.8	Locke + Mn 2.7 mM	S_2	22.0 ± 1.3
Locke	S_3	47.9 ± 4.5	Locke	S_3	57.0 ± 2.6
Locke + Mn 2.7 mM	S_4	9.0 ± 2.0	Locke + Mn 2.7 mM + Ca 10.5 mM	S_4	31.1 ± 1.9
Locke	S_5	39.9 ± 2.9	Locke	S_5	43.6 ± 3.2
Locke + Mn 2.7 mM	S_6	10.9 ± 2.0	Locke + Mn 2.7 mM + Ca 10.5 mM	S_6	38.4 ± 3.4
			Locke	S_7	49.8 ± 3.7

Each figure represents the mean acetylcholine output (with s.e. mean of 3 experiments) during the stimulation of the sympathetic trunk at 2 Hz for 5 min in consecutive samples during the perfusion of the superior cervical ganglion with Locke solution; Locke solution with addition of manganese 2.7 mM; Locke solution containing manganese 2.7 mM and high calcium, 10.5 mM.

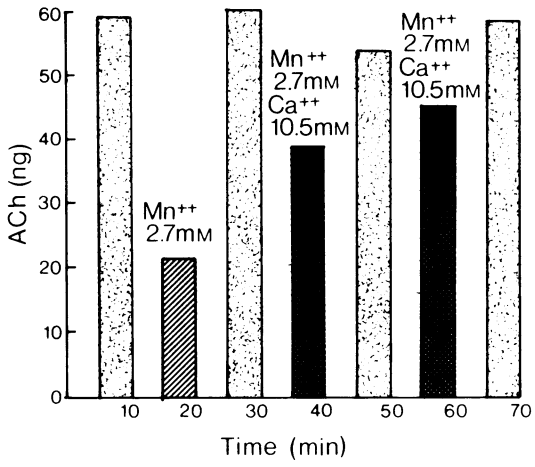

Fig. 5 Output of acetylcholine (ACh) from a ganglion perfused with Locke solution (physostigmine 10 μg/ml). Manganese (2.7 mM) was added to Locke solution in sample S_2 and to Locke solution with increased calcium (10.5 mM) in samples S_4 and S_6 (Table 1b). The change-over to the various perfusion fluids was performed 5 min before each stimulation period, exposing the ganglion to each solution for a period of 10 minutes. The columns represent the acetylcholine released during 5 min stimulation of the preganglionic nerve at 2 Hz followed by a 5 min resting period.

Discussion

In reducing the output of acetylcholine from the preganglionic nerve endings, manganese ions closely resemble magnesium ions whose antagonistic action to calcium ions on the transmitter release mechanism has long been recognized. (Hutter & Kostial, 1954; Del Castillo & Engbaeck, 1954). Since the present experiments with manganese followed the design of the original experiments with magnesium, an estimate of the relative potency of the two ionic species can be made. Thus, comparison with Figs 3 and 4, Hutter & Kostial (1954), shows that magnesium (25 mM), is about as effective as manganese (2.7 mM). Comparison is also possible with the results of Kostial & Vouk (1957) who studied the effect of lead ions on the output of acetylcholine and found the acetylcholine output to be reduced to about 25% by lead, 12.1 μM. As regards their potency in reducing acetylcholine output, these divalent cations may thus be ranked into the sequence magnesium $<$ manganese $\ll$ lead.

According to present knowledge, calcium influx into nerve endings is essential for the release of acetylcholine to occur (Katz, 1969). Direct proof that magnesium ions inhibit the influx of calcium ions into the desheathed superior cervical ganglion of the rat has recently been provided by Blaustein (1971). It seems highly likely that manganese ions act in a similar fashion, since it is already known that manganese ions reduce the slow inward calcium current in nerve and muscle fibres (Coraboeuf & Vassort, 1967; Baker, Hodgkin & Ridgeway, 1971).

The decrease in response to injected acetylcholine indicates that manganese ions have an action on the ganglion cells in addition to their action on the preganglionic nerve endings. On the present evidence the nature of the postganglionic change produced by manganese ions cannot be determined, but two possibilities can be envisaged. Firstly, on general grounds it might be expected that manganese ions share with other divalent cations the property of raising the threshold voltage for impulse propagation. Secondly, it seems possible that manganese ions produce a decrease in the chemosensitivity of the postjunctional membrane such as calcium and uranium oxide ions are known to produce at the motor end-plate (Nastuk & Liu, 1966). In this connection it might be noted that in the presence of calcium (10 mM) even high doses (up to 200 μg) of acetylcholine fail to produce contractions of the nictitating membrane when injected into the perfused superior cervical ganglion (Kostial & Landeka, unpublished). It may also be relevant to recall that low concentrations of uranium oxide ions decrease the acetylcholine sensitivity of ganglion cells to acetylcholine (Šlat & Kostial, 1959). Fatt & Katz (1952) have found that the amplitude of miniature end-plate potentials (m.e.p.ps) is reduced in the presence of high calcium ion concentrations. If manganese ions decrease the chemosensitivity of postjunctional membranes they should also reduce the amplitude of the m.e.p.ps. This is not borne out by the experiments of Meiri & Rahamimoff (1972), but these were done in low calcium concentrations to 'sharpen up' the prejunctional action of manganese, since the magnitude of the inhibition produced by manganese depends on the concentration of calcium in the extracellular medium, and involved concentrations of manganese much lower than those used here.

A feature of the present results was the reduction of the response to injected acetylcholine by lower concentrations of manganese than those required to decrease the response to preganglionic stimulation. The indication is that the quantity of acetylcholine normally released by nerve impulses at 2 Hz is greatly supraliminal. It may also be that the brief high frequency discharge of the ganglion cells produced by injected acetylcholine

(Takeshige & Volle, 1962) is especially sensitive to the post-ganglionic action of manganese and that it is abbreviated to such an extent that only negligible contractions of the nictitating membrane are produced. A more remote possibility, compatible with present evidence, is that the post-synaptic receptors acted upon by acetylcholine released from nerve endings are less sensitive to manganese ions than those accessible to injected acetylcholine.

The ready reversibility of both the pre- and post-junctional effect of manganese ions observed in these experiments is in keeping with the supposition that actions upon surface membranes are involved.

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