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A DUAL EFFECT OF COBALT IONS ON THE SPONTANEOUS RELEASE OF TRANSMITTER AT INSECT MOTOR NERVE TERMINALS

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SUMMARY

1. The effect of extracellular cobalt on the frequency of miniature excitatory post-synaptic potentials (MEPSPs) was studied in cockroach leg muscle fibres that had been depolarized with 20·8 mM-K saline.

2. Cobalt ions had a dual effect on the spontaneous release of transmitter, an inhibitory action being followed by an acceleratory. A reciprocal relationship between Ca^{2+} and Co^{2+} was found for both the inhibitory and acceleratory effects.

3. The equilibrium dissociation constant for Co^{2+} as a competitive antagonist of spontaneous release ranged from 0·4 to 0·65 mM. It is concluded that Co^{2+} is much more potent than Mg^{2+} in suppressing spontaneous transmitter release at the insect neuromuscular junction. The antagonism by extracellular Co^{2+} appears to occur only at the external surface site on the terminal membrane.

INTRODUCTION

Calcium ions are essential for the release of transmitter quanta at the neuromuscular junction (Katz, 1969) and this action of calcium is antagonized competitively by magnesium (Jenkinson, 1957; Dodge & Rahamimoff, 1967; Hubbard, Jones & Landau, 1968*b*). Recent studies have demonstrated that Co^{2+} (Weakly, 1973), Mn^{2+} (Meiro & Rahamimoff, 1972; Balnave & Gage, 1973) and La^{3+} (Blioch, Glagoleva, Liberman & Nenashev, 1968; Heuser & Miledi, 1971; DeBassio, Schnitzler & Parsons, 1972) are more effective than Mg^{2+} in suppressing evoked transmitter release. By contrast, these cations have been found to increase the spontaneous release of transmitter (Blioch *et al.* 1968; Heuser & Miledi, 1971; DeBassio *et al.* 1972; Kajimoto & Kirpekar, 1972; Balnave & Gage, 1973; Kita & Van der Kloot, 1973, Weakly, 1973). Therefore, one might expect that the mechanism of spontaneous release of transmitter is distinct from that of evoked transmitter release (Kajimoto & Kirpekar, 1972; Manalis & Cooper, 1973; Weakly, 1973; Hodgkis & Usherwood, 1978). To examine the possibility, I have compared the presynaptic effect of cobalt ions with that of magnesium in insect neuromuscular junctions that were partially depolarized with 20·8 mM-K saline, to elevate the level of spontaneous transmitter release. At this K concentration, the effect of divalent cations on the release was clearly demonstrated (Washio & Inouye, 1978).

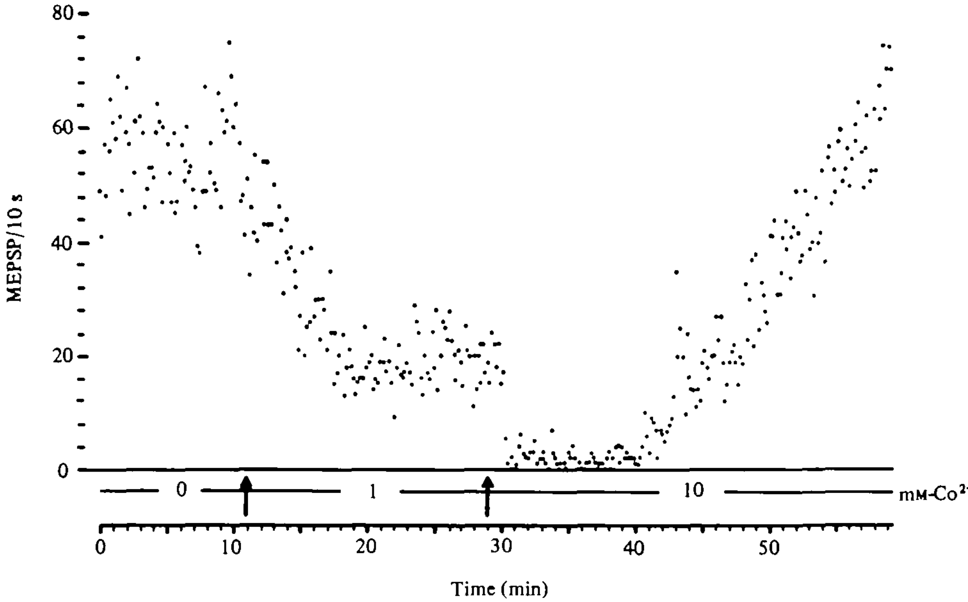

Fig. 1. Time course of the effect of external cobalt (1 and 10 mM) on MEPSP frequency in a single fibre in 20·8 mM-K saline containing 1 mM-Ca. Cobalt concentration was changed where indicated by arrows.

MATERIALS AND METHODS

Intracellular recordings of miniature excitatory post-synaptic potentials (MEPSPs) were made from coxal depressor muscles 178 and 136 (Carbonell, 1949) isolated from meta- and mesothoracic legs, respectively, of the cockroach, *Periplaneta americana*. The properties of the muscles have been described previously (Pearson & Iles, 1971; Washio & Inouye, 1978). The methods were similar to those used by Washio & Inouye (1978). The standard bathing solution had the following composition (mM): NaCl 158, KCl 10·8, $CaCl_2$ 5, HEPES 5. The pH of the solution was adjusted to 7·0 with NaOH. Test solutions were prepared by substituting NaCl with the chloride of the appropriate cation. The preparation was maintained in the standard saline for about 2 h and then transferred to a test solution containing 20·8 mM-K. Further changes in cation concentration were then made at this elevated K level. Recordings of MEPSP frequency were made when the frequency had reached a steady level. The preparation was perfused by constant flow at a rate of 5·0 ml/min. Experiments were all done at 20–24 °C.

RESULTS

MEPSP frequency, in the presence of 1 mM-Ca and 20·8 mM-K, was depressed after cobalt was applied. As cobalt concentration was raised there was an increase in the rate and in the magnitude of the depression (Fig. 1 and 2). The rate of depression was also increased when higher frequencies were observed in cobalt-free saline (Fig. 2). The depression of frequency induced by cobalt was reversible. The depression

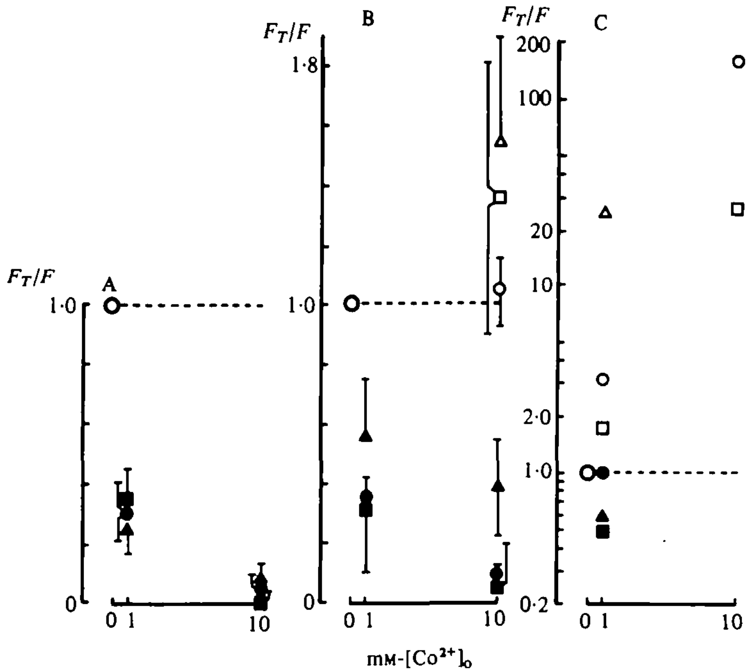

Fig. 2. Effect of external cobalt concentration on MEPSP frequency in 20·8 mM-K saline containing Ca at 5 mM (A), 1 mM (B) and 0·1 mM (C). The frequency on the ordinate is expressed as the ratio of the frequency in the particular test solution (F_T) to the frequency (F) in Co-free saline per 10 s. Each symbol in A, B, and C indicates mean frequency obtained from one muscle fibre. Filled symbols show the inhibitory effect and open symbols show the acceleratory effect of Co^{2+}. The data for 0·1 mM-Ca (C), plotted on a logarithmic scale, are from fibres in Table 1. Vertical bars indicate ± S.D. of each mean.

Table 1. *Effect of external cobalt concentration on MEPSP frequency in 20·8 mM-K saline containing* 0·1 *mM-Ca*

Muscle	Control	1 mM-Co: Initial decrease	1 mM-Co: Delayed increase	10 mM-Co
1(▲, △)	1·21 ± 1·14 (74)	0·72 ± 0·80 (60)	30·1 ± 6·1 (32)	—
2(■, □)	0·81 ± 0·92 (75)	0·39 ± 0·66 (60)	1·43 ± 0·92 (30)	22·4 ± 5·8 (30)
3(●, ○)	0·20 ± 0·40 (60)	0·20 ± 0·48 (60)	0·64 ± 0·58 (60)	32·0 ± 5·3 (60)

The MEPSP frequency per 10 s was compared in the same fibre in various concentrations of cobalt Results given as mean ± S.D. (n). Symbols correspond to those in Figure 2C.

could be followed by an increase if the concentration of cobalt was raised sufficiently (Fig. 1) at relatively low calcium concentrations (Fig. 2 and Table 1). The delayed increase in frequency began gradually about 15 min after elevating the external cobalt concentration to 10 mM in most preparations, the frequency eventually rising above the initial control level. There was an increase in the rate of the delayed increase as the cobalt concentration was raised. The increase in frequency was found to be partially reversible.

As calcium concentration was raised while keeping cobalt concentration constant,

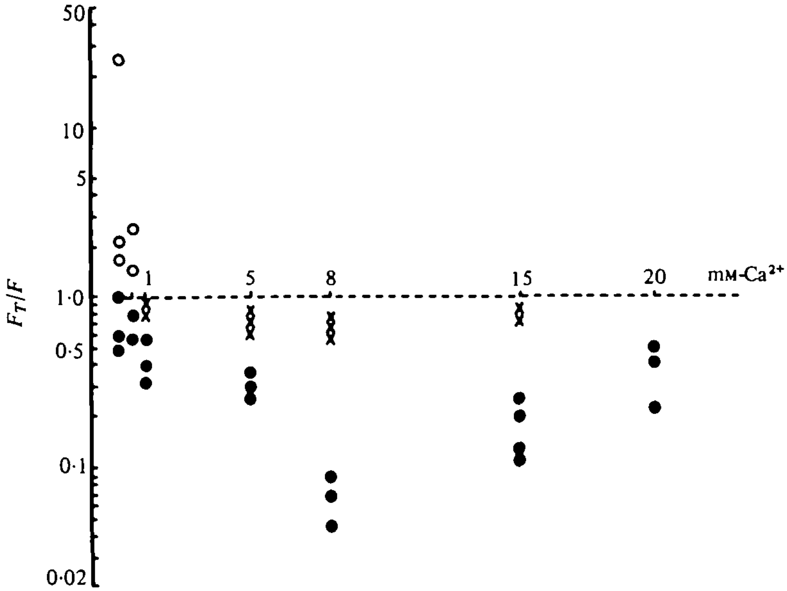

Fig. 3. Effect of calcium concentration on the acceleration and depression of MEPSP frequency by 1 mM-Co (○, ●) or 1 mM-Mg (×). Ordinate, MEPSP frequency per 10 s as the ratio of the frequency in the particular test solution (F_T) to the frequency (F) in Co-free or Mg-free salines on a logarithmic scale. Each data point was obtained from an individual fibre. A few pairs of points for initial depression (●) and delayed increase (○) were obtained from 3 and 2 fibres tested in the presence of 0·1 and 0·5 mM calcium saline, respectively.

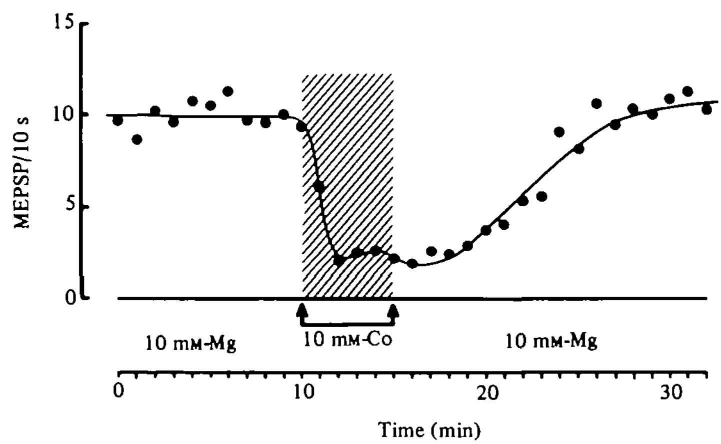

Fig. 4. A comparison of the effect of 10 mM-Mg^{2+} and 10 mM-Co^{2+} on the MEPSP frequency. The K^+ concentration was at 20·8 mM. The total divalent cation concentration was kept constant at 11 mM, Ca^{2+} concentration being 1 mM.

the initial depression of frequency was enhanced and the delayed increase was abolished (Fig. 3). The depression of MEPSP frequency by 1 mM-Co was at a maximum close to 8 mM calcium (Fig. 3). The effect of magnesium was compared to that of cobalt at a concentration of 1 mM over a range of calcium concentration (Fig. 3), and at a concentration of 10 mM (Fig. 4). Cobalt can be seen to be a more effective blocker than magnesium.

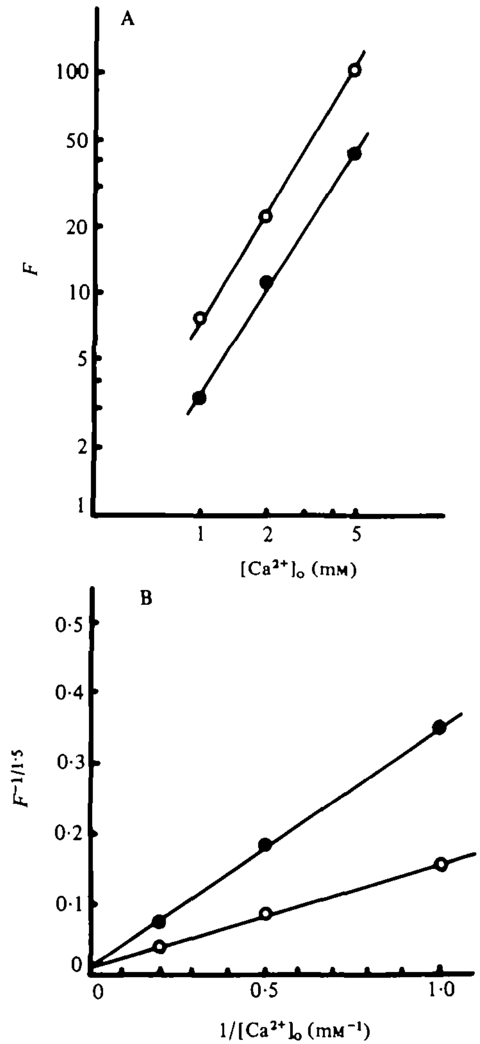

Fig. 5. The competitive interaction of calcium and cobalt in spontaneous transmitter release in elevated K^+ (20·8 mM) saline. (A) Double logarithmic plots of MEPSP frequency per 10 s (*F*, ordinate) against the external calcium concentration in the absence (○) and presence (●) of 0·5 mM-Co ions. (B) Modified Lineweaver-Burk plot of data shown in A. In the absence (○) and presence (●) of 0·5 mM-Co ions.

The antagonism by calcium of the inhibitory effect of cobalt suggested that these two cations could be competing for a common site upon the presynaptic membrane. This idea was supported by log-log plots of MEPSP frequency against calcium concentration. In the absence of cobalt, the slope of the relationship was 1·5 in Fig. 5A and varied from 0·5 to 2·4 in ten preparations. The slope was much less than the value obtained for the relation between end-plate potential amplitude and extracellular calcium concentration (Jenkinson, 1957; Dodge & Rahamimoff, 1967; Balnave & Gage, 1973; Jan & Jan, 1977), but comparable to the value obtained in the relation between miniature potential frequency and extracellular calcium concentration at mouse (Cooke, Okamoto & Quastel, 1973) and frog (Kita & Van der Kloot,

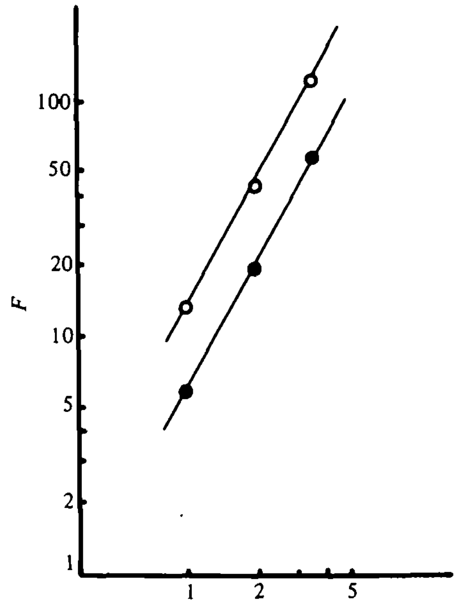

Fig. 6. The competitive interaction of calcium and magnesium in spontaneous transmitter release in elevated K^+ (20·8 mM) saline. Double logarithmic plots of MEPSP frequency per 10 s (*F*, ordinate) against the external calcium concentration in the absence (○) and presence (●) of 2 mM-Mg ions.

1973) neuromuscular junctions. In the presence of 0·5 mM-cobalt, the slope remained the same, but frequency was depressed (Fig. 5A). Plots of the reciprocal of the 1·5th root of the frequency against the reciprocal of the external calcium concentration (after Lineweaver-Burke, 1934) were linear in the presence and absence of cobalt, but were steeper when cobalt was present. The intercept on the ordinate remained unchanged (Fig. 5B).

The above results are taken as evidence that Ca^{2+} and Co^{2+} compete for a common site as has been postulated for the action of Co^{2+} on the evoked transmitter release (Weakly, 1973). The equilibrium dissociation constant (K_{II}) for Co^{2+} with the hypothetical membrane complex, CoX (analogous to MgX; del Castillo & Katz, 1954), as a competitive antagonist of spontaneous release was calculated from the relation (Jenkinson, 1957; Dodge & Rahamimoff, 1967; Weakly, 1973):

$$K_{II} = \frac{[Co]}{\frac{[Ca_2]}{[Ca_1]} - 1}$$

where $[Ca_1]$ is the concentration of Ca^{2+} that produces a certain frequency of MEPSPs and $[Ca_2]$ is the concentration of Ca^{2+} that produces an identical frequency of MEPSPs in the presence of 0·5 mM-Co^{2+}. For the experiment shown in Fig. 5A MEPSP frequency in the presence of 1 mM-Ca^{2+} was equal to that in about 1·9 mM Ca^{2+} with 0·5 mM-Co^{2+}. From the equation K_{II} was 0·55 mM. K_{II} for Co^{2+} range from 0·40–0·65 mM in four experiments of this type with a mean of 0·52 mM. The value is similar to that obtained for Co^{2+} acting as an antagonist of synchronous (Weakly, 1973; Crawford, 1974) and asynchronous (Silinsky, Mellow & Phillips, 1977) evoked transmitter release by nerve stimulation.

A double logarithmic plot of MEPSP frequency for the antagonism by Ca of the effect of 2 mM-Mg (Fig. 6) yielded a K_{Π} value of 4·0 mM. This confirms that Co^{2+} is much more potent than Mg^{2+} in suppressing spontaneous transmitter release.

DISCUSSION

The present experiments have shown that cobalt ions have a dual effect on the spontaneous release of transmitter at the insect motor nerve terminal. Increasing cobalt concentration always depressed MEPSP frequency, but at relatively low calcium concentrations this was followed by an increase in frequency (Fig. 2 and 3). Increasing calcium concentration depressed both inhibitory and acceleratory effects of Co^{2+} (Fig. 3). A similar reciprocal relationship between Ca^{2+} and Mg^{2+} has been found in vertebrate (Hubbard *et al.* 1968*a*; Blioch *et al.* 1968) and invertebrate (Washio & Inouye, 1978) nerve terminals. The reciprocal relationship between the effect of Co^{2+} and Ca^{2+} on spontaneous transmitter release may be accounted for by a competitive antagonism between them for common receptor sites at the motor nerve terminals, on a similar manner to the antagonism between Ca^{2+} and Co^{2+} on the evoked release (Weakly, 1973). In addition, Co^{2+} has been shown to be a competitive antagonist for spontaneous release of transmitter following tetanus (Silinsky *et al.* 1977). Thus, both evoked and spontaneous release of transmitter may share common receptor sites. The receptor for spontaneous release may be at the external surface of the terminal, as indicated for a receptor antagonized by calcium and magnesium (Kharasch, Mellow & Silinsky, 1981).

The inhibitory effect of Co^{2+} does not appear to be due to electrostatic screening of fixed charges (Matthews & Wickelgren, 1977; Madden & Van der Kloot, 1978), because the effects could be observed when the total concentration of divalent cations was kept constant (Fig. 4). Furthermore, MEPSP frequency had been observed to increase as calcium concentration is increased up to 10 mM in 20·8 mM-K saline at insect neuromuscular junctions (Washio & Inouye, 1978). However, as calcium concentration is increased above 10 mM, MEPSP frequency declines (Washio unpublished observation).

Although the mechanism responsible for the delayed accelerating action of Co^{2+} remains to be defined, we may envisage that cobalt may enter a nerve terminal, depolarized by the elevated K^{+}, through the calcium pathway. Once within the terminal Co^{2+} may stimulate the quantal release of transmitter (Kita & Van der Kloot, 1976; Kita, Narita & Van der Kloot, 1981), possibly by causing an increase in the intracellular free calcium concentration as a result of interfering with the internal calcium store (Miledi & Thies, 1971; Baker, 1972; Alnaes & Rahamimoff, 1975). It should also be noted that cobalt can induce a prolonged but reversible increase in membrane permeability to calcium in the acinar cells of cockroach salivary glands (Mitchell, Ginsborg & House, 1980).

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