Albumin inhibits effects of Cu^{2+} on autonomic postganglionic transmission of guinea-pig ileum and vas deferens

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The influence of albumin, 4.5 and 45 g/l, on the effects of Cu^{2+}, 10^{-9}–10^{-3} M, on the neuromuscular transmission of the isolated guinea-pig ileum and vas deferens was investigated. Low concentrations of Cu^{2+}, 10^{-9}–10^{-6} M, caused a slight and transient increase of the contractile response to direct muscle stimulation of the vas deferens. Albumin at 4.5 g/l inhibited this effect. The basal tone of the ileum increased transiently when Cu^{2+} was added in the presence of albumin, 4.5 g/l. A stimulant action of Cu^{2+}, 10^{-9}–10^{-6} M, was seen on both organs, when contractions were induced by nerve stimulation. All these effects were inhibited by albumin, 45 g/l. Higher concentrations of Cu^{2+}, 10^{-5}–10^{-4} M, increased the basal tone of the ileum and the vas deferens. In the presence of albumin, 45 g/l, this stimulating effect of Cu^{2+} appeared in higher concentrations. The results suggest that Cu^{2+} is more likely to influence the function of neuronal tissues when the concentration of protein is low.
☐ *Amalgam; copper; neuro-muscular transmission; smooth muscle*

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Copper is a constituent of most amalgam alloys and many gold alloys used in restorative dentistry. The corrosion of Cu phases in amalgams, especially in the new copper-rich type, is associated with a substantial release of copper (1–2). Metallic taste, burning sensations in the tongue and mucous membrane, and other mucosal symptoms have been associated with amalgam and gold restorations (3–6). These symptoms might be attributable to the action of Cu, released from the restorations, on excitable tissues in the mucous membrane.

A previous study (7) showed that Cu^{2+}, in low concentrations of 10^{-8}–10^{-6} M (0.6–60 ng/ml), had an excitatory effect on autonomic cholinergic neuromuscular transmission. Albumin has been shown to reduce the effects of Cu^{2+} on biologic tissue (8) but did not reduce the inhibiting effect of inorganic Hg^{2+} on autonomic neuromuscular transmission (9). The aim of this study was therefore to investigate the possible influence of albumin on effects exerted by Cu^{2+}, 10^{-9}–10^{-3} M, on cholinergic and adrenergic neuromuscular autonomic transmission.

Materials and methods

Mottled male guinea-pigs (250–500 g) were killed with a blow on the head. Segments (2–3 cm) from the distal ileum and the pair of vas deferens were suspended in organ baths described previously (9) and connected to recording transducers (Grass FT 03C). The transducers were coupled to a polygraph (Grass model 7B). The bath solution, 44 ml, was recirculated (2 ml/min) in a closed system (perfusion, 37°C) and aerated with 6.5% CO_2 in O_2. Before the start of experiments the preparations were allowed to equilibrate in the bath solution for at least 60 min.

The effect of Cu^{2+} on the preparations immersed in ordinary Tyrode solution was studied. The composition of Tyrode solution

Table 1. Effects of Cu^{2+} (in molar) on the basal tone and contractile response of isolated guinea-pig ileum and vas deferens in the presence of albumin

	Ileum								Vas deferens							
	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	*n*	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	*n*
Basal tone																
Tyrode solution	0	0	0	0	+	++	+++	24	0	0	0	0	0	+	+++	24
+ Albumin, 4.5 g/l	+	+	+	++	++	++	+++	12	0	0	0	+	+	+	+++	13
+ Albumin, 45 g/l	0	0	0	0	0	+	+++	18	0	0	0	0	0	0	0	14
Muscle stimulation																
Tyrode solution	0	0	0	0	0	−−	−−	10	+	+	+	++	++	++	++	11
+ Albumin, 4.5 g/l	0	0	0	0	0	0	+/−−−	6	0	0	0	+	+	+	+++	6
+ Albumin, 45 g/l	0	0	0	0	0	0	−−−	6	0	0	0	0	++	++	++	8
Nerve stimulation																
Tyrode solution	+	+	+	+	+	−−−	−−−	14	+	+	+	+	+	++	++	13
+ Albumin, 4.5 g/l	0	0	0	0	0	0	−−−	6	+	+	+	+	+	+	+++	7
+ Albumin, 45 g/l	0	0	0	0	0	−	−−	12	0	0	0	0	0	0	+++	6

Increase is indicated by +, decrease by −, and no effect by 0. Preparations responding to increasing concentration of Cu^{2+} are cumulatively given as + (< 25% of the preparations), ++ (25–75%), +++ (> 75%), and − (< 25%), −− (25–75%), −−− (> 75%). +/− indicates increase followed by decrease.

was 136.7 mM NaCl, 2.7 mM KCl, 11.9 mM $NaHCO_3$, 1.8 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.3 mM $NaHPO_4$ (all analytical grade; E. Merck) and 5.6 mM glucose (BDH) dissolved in deionized and double-distilled water.

The effect of Cu^{2+} was further studied in Tyrode solution containing albumin, 4.5 g/l and 45 g/l. The bath solution compositions and the electric stimulation of the preparations have been described in detail previously (9).

Fresh stock solutions were prepared with $CuCl_2$ dissolved in the bath medium, which was added to an initial concentration of 10^{-9} M (0.2 ng/ml) in the bath solution. The bath solution was pumped through the organ bath, and the action of Cu^{2+} on the contractility was recorded. A criterion for an effect clearly attributable to Cu^{2+} was a change of more than 20% of the initial contractile response to electric stimuli. The concentration of $CuCl_2$ in the organ bath was repeatedly increased 10-fold by new additions (0.2 ml) of $CuCl_2$ solutions. The concentration of ionized Ca^{2+} in the bath solution was monitored throughout the experiment and was stable around 1.5 mM. Preparations with high spontaneous activity were excluded from the study. Each type of experiment was performed on 6–24 preparations. Four ileum and two vas deferens preparations were taken from each guinea-pig.

Results

The results are summarized in Table 1.

Response of the smooth muscle

Effects of Cu^{2+} on the basal tone and on the contractile response to direct muscle stimulation. Cu^{2+}, 10^{-9}–10^{-6} M, in ordinary Tyrode solution caused a slight and transient increase of the contractile response by the vas deferens (Fig. 1A). This was not seen in the ileum (Fig. 2A). Cu^{2+}, 10^{-5} M, raised the basal tone in the ileum for 5–15 min, followed by relaxation. Coincidentally with the rise in tone, Cu^{2+}, 10^{-4}–10^{-3} M, gradu-

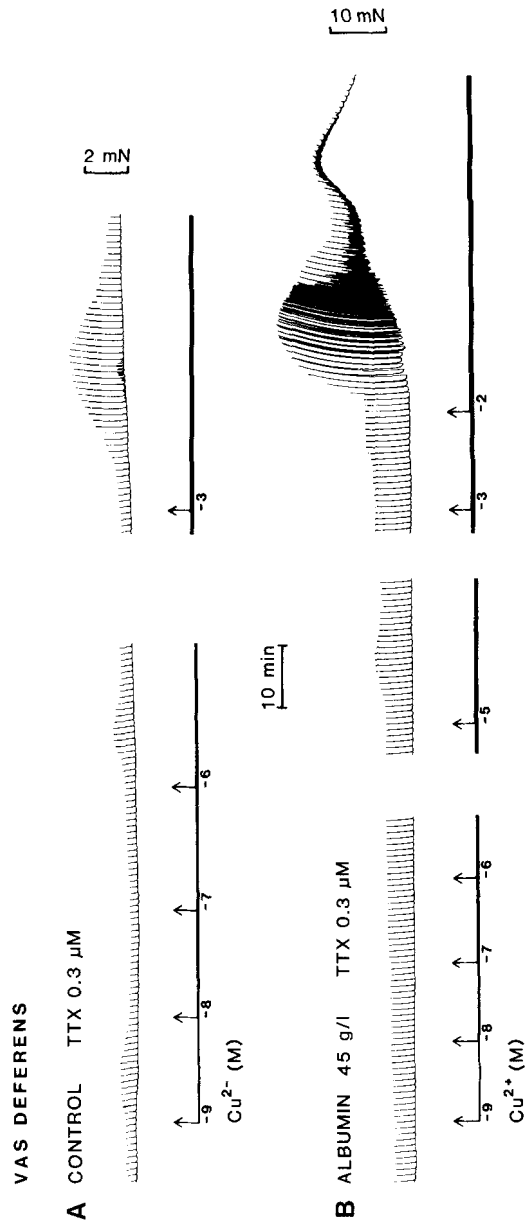

Fig. 1. Cu^{2+} effects on field-stimulated (direct muscle stimulation) guinea-pig vas deferens (Cu^{2+} concentrations in 10 logarithm). TTX = tetrodotoxin. 1A. Cu^{2+}, 10^{-9} and 10^{-6} M, slightly increased the response to electric stimulation. Cu^{2+}, 10^{-3} M, raised the basal tone and sharply increased the contractile response to electric stimulation. 1B. Albumin, 45 g/l, inhibited the stimulant effect of Cu^{2+} in low concentrations (10^{-9}–10^{-6} M). Higher concentrations, 10^{-5} M, slightly increased the contractile response to electric stimulation. Cu^{2+}, 10^{-2} M, had to be added to increase the basal tone.

ally decreased the strength of the ileal contractions, whereas those of the vas deferens increased.

Influence of albumin on the effects of Cu^{2+}. When albumin, 4.5 g/l, was present, Cu^{2+} in low concentrations, 10^{-9}–10^{-6} M, transiently (5–15 min) increased the basal tone in the ileum (Fig. 2B). However, in the vas deferens the enhancement of the response to electric stimulation (see above) was inhibited (Fig. 1B). Higher concentrations of Cu^{2+} had to be added to the bath to raise the basal tone when albumin was present (Fig. 1B, 2B, C). Albumin also counteracted the depressant effect of Cu^{2+} on the contractile response to electric stimulation in the ileum (Fig. 2B, C), whereas the stimulant effect on the vas deferens was not influenced.

Response to nerve stimulation

Effects of Cu^{2+}. In contrast to the contractile response to direct muscle stimulation Cu^{2+}, 10^{-9}–10^{-6} M, caused a slight increase

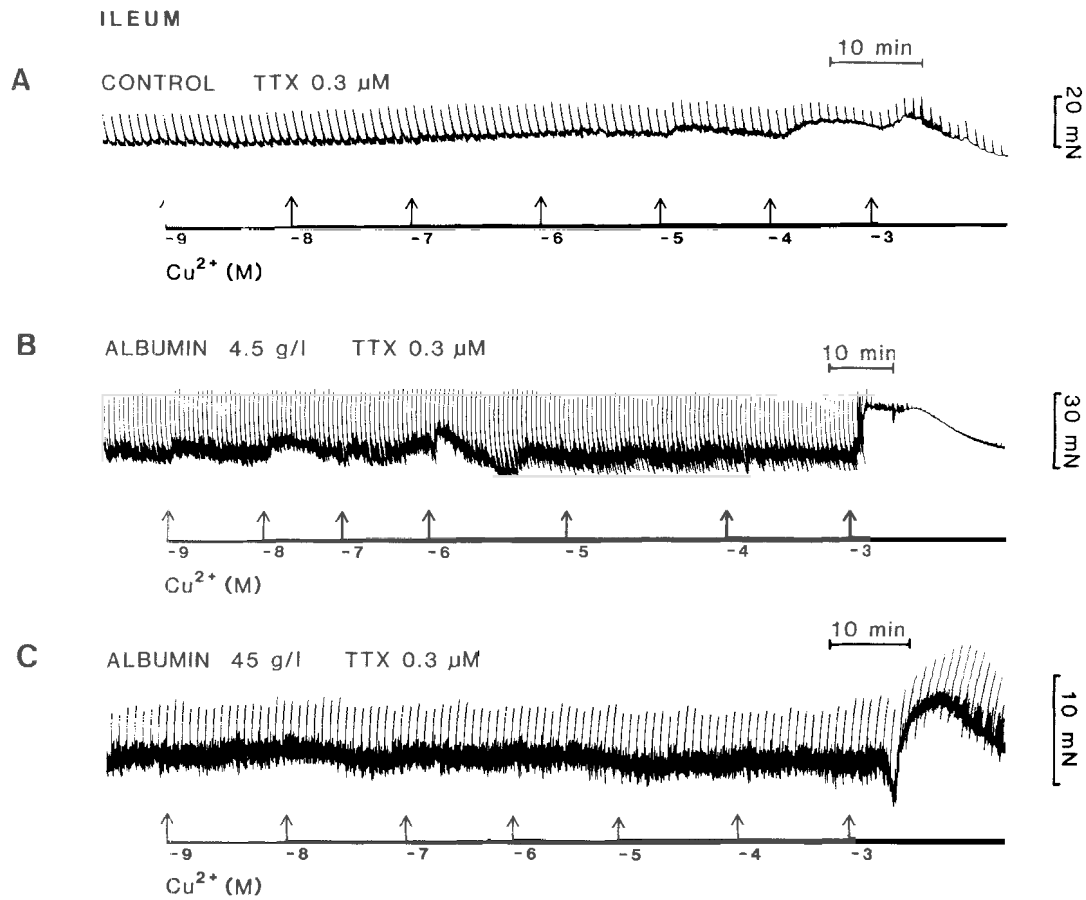

Fig. 2. Cu^{2+} effects on the field-stimulated (direct muscle stimulation) guinea-pig ileum (Cu^{2+} concentrations in 10 logarithm). TTX = tetrodotoxin. 2A. Cu^{2+} in low concentrations, 10^{-9}–10^{-6} M, had no effect on the response to electric stimulation. Higher concentrations, 10^{-5}–10^{-3} M, increased the basal tone, and the response to electric stimulation decreased. 2B. In the presence of albumin, 4.5 g/l, Cu^{2+}, 10^{-9}–10^{-6} M, transiently increased the basal tone of the ileum, and a higher concentration of Cu^{2+} had to be added to depress the response to electric stimulation. A sharp rise in tone was seen when Cu^{2+}, 10^{-3} M, was added, and the response to electric stimulation faded. 2C. Albumin, 45 g/l, inhibited the stimulant effect of Cu^{2+}, 10^{-9}–10^{-4} M. The basal tone increased sharply when Cu^{2+}, 10^{-3} M, was added and the depression of the response to electric stimulation caused by Cu^{2+} was delayed as compared with when albumin, 4.5 g/l, was present.

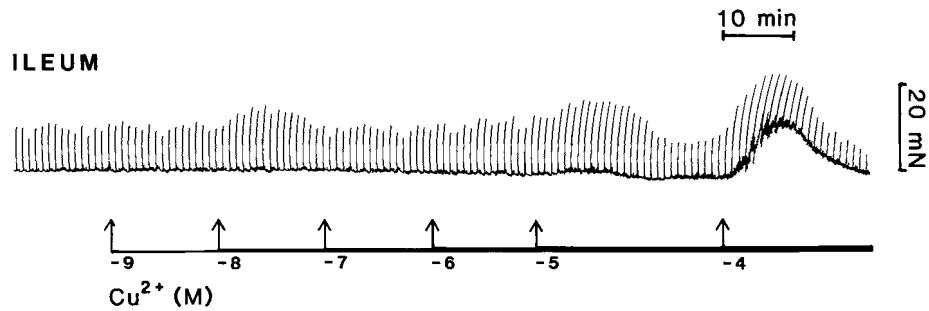

Fig. 3. Cu^{2+} effects on the field-stimulated (neurogenic) guinea-pig ileum (Cu^{2+} concentrations in 10 logarithm). Contrary to contractions induced by direct muscle stimulation, Cu^{2+}, 10^{-8} M, increased the response to nerve stimulation in ordinary Tyrode solution.

in the response of the ileum to nerve stimulation (Fig. 3). In the vas deferens the stimulant effect of Cu^{2+} on the response was similar to that of direct muscle stimulation. The response to higher concentrations of Cu^{2+}, 10^{-5}–10^{-3} M, was the same as recorded on direct muscle stimulation (above) in both the ileum and the vas deferens.

Influence of albumin on the effects of Cu^{2+}. Albumin blocked the stimulant effect of Cu^{2+}, 10^{-9}–10^{-5} M, on the contractile response to nerve stimulation of the ileum. However, Cu^{2+}, 10^{-9}–10^{-7} M, still had a stimulant effect on the contractile response of the vas deferens in the presence of albumin, 4.5 g/l. This stimulating effect was not seen on contractions induced by direct muscle stimulation.

Contrary to the contractions induced by direct muscle stimulation of the vas deferens, Cu^{2+} at the higher concentrations, 10^{-5}–10^{-4} M, had no stimulant effect in the presence of albumin, 45 g/l (Fig. 4).

Discussion

The present results indicate that Cu^{2+} in very low concentrations, 10^{-9}–10^{-6} M, has stimulant effects on the smooth muscles of guinea-pig ileum and vas deferens. This is in accordance with an earlier study on guinea-pig ileum (7). An effect of Cu^{2+} on an easily depleted or an easily deteriorated Ca^{2+} transport mechanism was proposed. This is consistent with the findings in the present study, as the stimulant effect of Cu^{2+} could not be reproduced unless a higher concentration was added. The sensitivity difference between the organs might be due mainly to differences in diffusion of Cu^{2+} in the tissue.

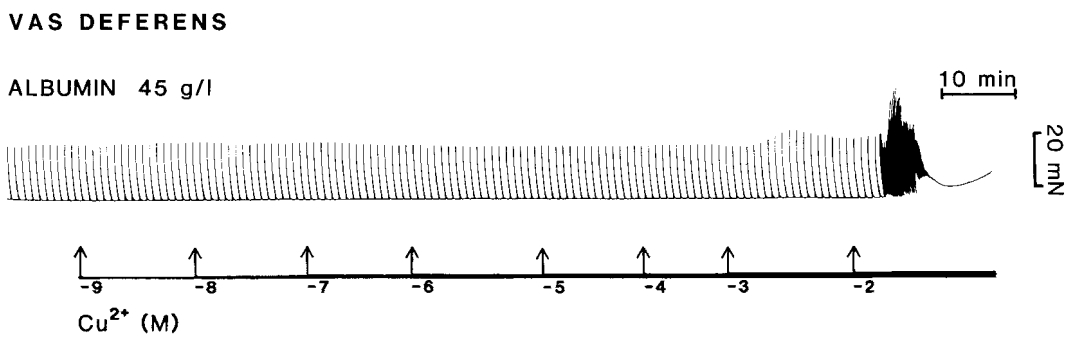

Fig. 4. Cu^{2+} effects on the field-stimulated (neurogenic) guinea-pig vas deferens (Cu^{2+} concentrations in 10 logarithm). When albumin, 45 g/l, was present, no effects of Cu^{2+} were seen on the response to electric stimulation until a concentration of 10^{-3} M was added.

A low concentration of albumin, 4.5 g/l, could augment the stimulant effects of Cu^{2+} on the ileum. A similar effect has been reported in an earlier study (7) of ileum in stagnant Tyrode solution, but without albumin. The stagnant bath solution might have enabled the secretion from the ileum to concentrate near the organ. Hence proteins in the secretion could interact with Cu^{2+} and increase the response, as in the present study with albumin. In the present study circulation of the bath solution spread and diluted the secretion of ileum in the entire bath, which might explain the absence of effect of Cu^{2+} in plain Tyrode solution. However, a high concentration of albumin (45 g/l) eliminated the stimulant effects of Cu^{2+} in low concentrations (10^{-9}–10^{-6} M). This could be due to binding of Cu^{2+} to albumin, reducing its ability to react with the organs. At higher concentrations of Cu^{2+} most binding sites of albumin might have been saturated, implying incapacity to bind excess of the metal. However, the stimulating effect of Cu^{2+} (10^{-5}–10^{-3} M) on the response to direct muscle stimulation in the vas deferens was not influenced by the albumin.

In the low concentration range, 10^{-9}–10^{-7} M, Cu^{2+} enhanced the contractile response to neurogenic stimulation (the ileum in plain Tyrode solution; the vas deferens in Tyrode solution with 4.5 g albumin/l). This might be due to a prejunctional stimulant action of Cu^{2+}, as no effect of Cu^{2+} was observed when contractions were induced by direct stimulation of the muscles. An alternate possibility is that Cu^{2+}, by a 'subliminal' action on the smooth muscle, enhanced the motor response to neurogenic stimulation (10, 11).

The concentration of copper in human saliva and in the mucous membrane near dental restorations (12) could approach the threshold for effects in the present study. The fraction of free Cu^{2+} ions and the proteins to which Cu^{2+} is bound in the oral cavity after corrosion are not known. Furthermore, when the quality and/or quantity of saliva is suboptimal, more Cu^{2+} may be released (2), whereas the amount of protein will decrease. The amount of free Cu^{2+} will therefore increase. Under these circumstances excitable tissue such as taste and pain receptors might be influenced by Cu^{2+}.

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