the 2nd part the effects of 800 mg were compared with 600 mg following a similar design to determine whether the effect previously observed depended on the dose. During each experimental session, the subjects' nociceptive reflex threshold was measured during the 5 min immediately preceding p.o. administration of the drug or placebo in order to determine the initial baseline (T0).

After drug administration the reflex was tested again between T30 and T60 in the 1st stage (1000 mg vs placebo) and between T30 and T90 in the 2nd stage then, between T130 and T180 in both stages. At T150, in the 2nd stages, the subjects were given an injection of naloxone (0.8 mg i.v.) to test whether the drug effect could be modified by this specific narcotic antagonist[7].

The numerical data of the threshold of the nociceptive reflex were analyzed separately for the 2 stages according to the method described by Wallenstein and Fisher[8]. The analysis indicated no overall carry over effects. The interactions treatment × time were then tested in each of the 2 stages and following by individual comparisons.

The figure shows that oxapadol at the 3 doses tested increased the nociceptive flexion threshold, whereas placebo was without effect. Between oxapadol 1000 mg and placebo the difference became significant ($p \leqslant 0.05$) at T40. In the 2nd part of the session (T130–T180) the increase of the threshold was stable and the difference between oxapadol and placebo was highly significant ($p \leqslant 0.01$).

The results obtained with oxapadol at 800 mg are similar to those obtained with 1000 mg but 600 mg are slightly more active; a significant difference ($p \leq 0.05$) between 600 mg and 800 mg is apparent at T70.

These results are similar to those obtained with other non-narcotic analgesics[4,9,10] (acetylsalicylic acid, glaphenine).

No subjects reported any undesirable side effects such as drowsiness, nausea or dyspnea at doses which markedly attenuated the nociceptive reflex.

Naloxone when injected at T150, where the inhibition of the nociceptive reflex was maximal, was without effect. It suggests that oxapadol-induced inhibition of pain sensation does not result from morphine-like activity.

In conclusion, oxapadol, a chemically original compound which shows analgesic activity in animal tests, reduces experimentally-induced pain in normal human subjects. These results together with preliminary clinical findings suggest that oxapadol may represent a new type of non-narcotic analgesic.

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A novel effect of cobalt treatment on calcium-dependent responses of the cockroach salivary gland

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Summary. After incubation in calcium-free solutions containing cobalt, the readmission of calcium caused prolonged but reversible hyperpolarization of acinar cells of cockroach salivary glands and prolonged fluid secretion. It is suggested that cobalt treatment increases the permeability of the acinar cell membrane to calcium.

The presence of cobalt ions is known to inhibit the release of neurotransmitter from nerve terminals[2] and the generation of calcium dependent action potentials[3]. These effects are generally believed to result from the inhibition by cobalt of calcium entry into cells[4]. Cobalt also inhibits fast axoplasmic flow[5] and augments the spontaneous release of neurotransmitter[2].

These effects have been taken to imply that cobalt enters cells and displaces calcium from intra-cellular binding sites[4]. We report here a novel effect of cobalt treatment, namely the enhancement of calcium dependent electrical and secretory responses of an exocrine gland, which suggests that cobalt can induce a prolonged but reversible increase in membrane permeability to calcium.

The electrical experiments were made on isolated salivary glands of *Nauphoeta cinerea* (Olivier)[6] bathed in flowing solution containing (mM) NaCl, 160; KCl, 1; Tris-HCl pH 7.6 buffer 5. Usually the control solution contained 5 mM $CaCl_2$ and the conditioning solutions had no added calcium and either 5 mM $CoCl_2$ or 5 mM $MgCl_2$ or 1 mM $MgCl_2$ plus 1 mM EGTA; modifications are mentioned below. Cells were impaled with microelectrodes containing 3 M potassium acetate.

Figure 1,a illustrates the transient hyperpolarization which occurred when the preparation was re-exposed to a calcium-containing solution after a period of exposure of about 15 min to a calcium-free solution[7]. 7 such responses were recorded: in 6 the duration was less than 3 min, and in the 7th less than 5 min. Figure 1,b illustrates the large prolongation of the response to the readmission of calcium after a period of exposure to a calcium-free solution containing 5 mM cobalt. Such a response occurred in more than 20 experiments.

In some of these the duration could not be determined because the electrode was dislodged before the resting potential had returned to its control value but in several it was clear that some degree of hyperpolarization remained 1 h after the readmission of calcium. The effect was reversible and could be obtained more than once in the same preparation. The hyperpolarization was not abolished

by concentrations of phentolamine which prevent hyperpolarization responses to intense nerve stimulation or dopamine application and is thus not likely to be due to transmitter released from the nerve terminals[8]. However it was found in separate experiments that like the responses to nerve stimulation or applied dopamine[9], the calcium readmission responses are associated with a reduction in membrane resistance and their amplitude is steeply dependent on the concentration of potassium in the bathing fluid. It therefore seems reasonable to suppose that the responses are due to an increase in potassium permeability of the acinar cell membrane resulting from an increase in the intracellular concentration of ionized calcium[10,11]. Thus, 2 possible explanations for the effect of cobalt are a) that it causes a prolonged inhibition of intracellular calcium binding, so allowing the prolongation of the effect of any particular calcium influx or b) that it causes a prolonged increase in calcium permeability. That process b) must make an important contribution is shown by the fact that the amplitude of the hyperpolarization depended on the concentration of calcium in the bathing fluid. As illustrated in figure 2 the response could be rapidly reversed by the removal of extracellular calcium and reinstated by its readmission.

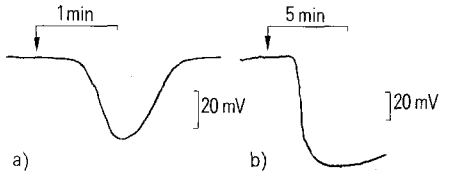

Fig. 1. *a* Effect on membrane potential of acinar cell of readmitting control solution containing 5 mM $CaCl_2$ to a preparation exposed to a Ca-free solution containing 5 mM $MgCl_2$ for the preceding 13 min. There was no change in resting potential during exposure to the Ca-free solution. *b* Effect of readmitting control solution containing 5 mM $CaCl_2$ to a preparation exposed to 5 mM $CoCl_2$ for the preceding 15 min. Phentolamine (5×10^{-4} M) present. A depolarization of 7 mV occurred during exposure to the Co-containing solution. The original resting potentials were about −40 mV.

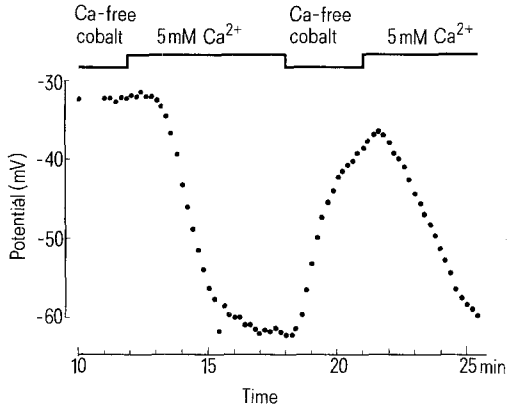

Fig. 2. Effect of withdrawal of calcium on the readmission response. Abscissa, time after beginning of exposure to. cobalt; Ordinate, membrane potential.

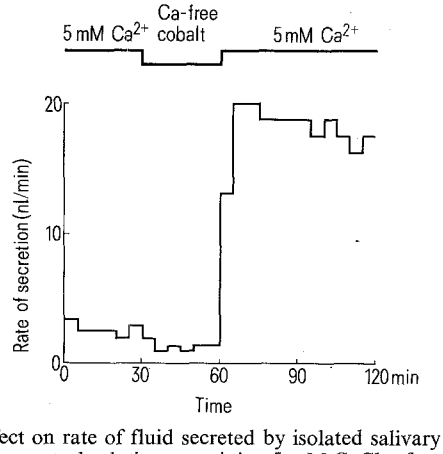

Fig. 3. Effect on rate of fluid secreted by isolated salivary gland of readmitting control solution containing 5 mM $CaCl_2$ after exposure to Ca-free solution containing 5 mM $CoCl_2$. Both solutions contained 10 mM KCl and 10^{-4} M phentolamine but were otherwise as for the electrical experiments.

No readmission response occurred if calcium was also present during the cobalt incubation period nor was there any change in resting potential. This suggests that calcium competes with cobalt for the site at which cobalt acts.

The secretory responses of the isolated gland were monitored by measuring the volume of fluid emerging from the ducts as previously described[12]. Although, as was shown by Douglas and Rubin[13], an impressive secretion of catecholamines from the adrenal medulla occurs on readmission of calcium, only transient secretory effects have hitherto been reported for exocrine glands[14,15]. In the present experiments there was little if any enhancement of fluid secretion above the basal rate when the control solution was readmitted after a preceding exposure to a test solution containing either no divalent cation or 5 mM magnesium. After exposure to a solution containing 5 mM cobalt, however, the readmission of control solution gave rise to a large prolonged increase in fluid secretion, as illustrated in figure 3. The analogous electrical and secretory responses suggest that both may result from an increase in intracellular ionized calcium[16,17] but the absence of a secretory response to correspond with the transient electrical response after magnesium-treatment suggests that the electrical and secretory processes may have different quantitative requirements for calcium. Further work on the readmission responses is in progress.

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