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Letter to the Editor

Acute treatment with clonidine or calcium entry blockers on opioid withdrawal in guinea-pig ileum

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It is generally accepted that the typical symptoms of morphine withdrawal are caused by a rebound neuronal hyperactivity in the central and peripheral nervous system. The morphine abstinence symptoms can be effectively suppressed by administering clonidine, which decreases noradrenaline (NA) release in brain (Taylor et al 1988) and has been used in the treatment of opioid withdrawal in man (Washton & Resnick 1980). The main effect of clonidine on the withdrawal syndrome seems to be mediated by the decrease of NA release in the locus coeruleus. Nevertheless, clonidine also induces peripheral effects. Collier et al (1981) showed that clonidine could suppress the effects of normorphine withdrawal in in-vitro experiments with guinea-pig ileum, probably caused by a decreased release of acetylcholine from the cholinergic motor neuron. The in-vitro withdrawal could be evoked in myenteric plexus-longitudinal muscle (MP-LM) strips obtained from morphine-dependent guinea-pigs. In those tissues, naloxone induces a withdrawal sign consisting of an abrupt contracture produced by an excessive release of acetylcholine (Frederickson et al 1976; Schulz et al 1985). Furthermore, concomitant electrophysiological events recorded in myenteric neurons have been demonstrated (Johnson et al 1987), providing direct evidence of the naloxone-induced depolarization of the neuronal membrane.

On the other hand, after chronic morphine treatment, the binding of $^{45}Ca^{2+}$ to synaptic vesicles was augmented and a large increase in the content of calcium in the brain was found; during the naloxone-precipitated withdrawal syndrome these elevated levels of calcium return toward control values (Yamamoto et al 1978). Furthermore, calcium channel blocking drugs reduced the occurrence of behavioural signs of naloxone-precipitated morphine withdrawal (weight loss, diarrhoea, wet dog shakes, grooming, teeth chattering) (Bongianni et al 1986; Baeyens et al 1987); however, the mechanism underlying that effect of the calcium channel blocking drugs has not been elucidated and central and peripheral sites of action may be implicated, especially for the suppression of the body weight loss and diarrhoea. In MP-LM strips of guinea-pig ileum calcium entry blocking drugs antagonize the contractile response induced by electrical stimulation, an effect which can be attributed to a decrease of the acetylcholine release by a blockade of calcium influx through potential operated channels, or to an inhibition of the calcium entry into smooth muscle cells (Llinas 1982; Martin et al 1988).

To compare and evaluate the peripheral antiwithdrawal effects of clonidine with that of calcium entry blocking drugs, the frequency of presentation of the contractile response elicited by administration of naloxone was studied in isolated ileum from guinea-pigs chronically treated with morphine.

The effect of naloxone was tested on the MP-LM strips obtained from: (1) control animals, (2) non-dependent animals, treated with clonidine, diltiazem or nimodipine, (3) dependent animals, (4) dependent animals treated with clonidine, diltiazem or nimodipine. Clonidine (75 $\mu g\ kg^{-1}$), diltiazem (10 mg kg^{-1}) or nimodipine (5 mg kg^{-1}) was given i.p. 1 h before the animals were killed. The effect of naloxone was also tested on morphine-tolerant MP-LM strips incubated with clonidine (10 nM), diltiazem (100 nM) or nimodipine (25 nM).

Morphine-dependence was obtained by administration of a single subcutaneous injection of a suspension of morphine four days before the animals were killed (Frederickson et al 1976).

The naloxone-induced contraction was regarded as positive when the contractile force was 0·5 g and negative when no contraction was observed. Responses between 0 and 0·5 g were discarded. Statistically significant differences were determined by the Chi^2 test and by Student's *t*-test. Data are expressed as mean ± s.e.m.

The administration of naloxone (1 μM) to the organ bath induced contractile responses in 55% (n = 10) of the MP-LM strips isolated from morphine-treated animals. The contractile response was absent in ileum segments from naive animals (n = 10). When morphine-dependent guinea-pigs were pretreated with clonidine, nimodipine or diltiazem, or when MP-LM preparations from morphine-dependent animals were incubated with the drugs, the frequency of the naloxone-induced contractions was significantly decreased (Fig. 1).

On the basis of results obtained in pilot experiments, the in-vitro concentrations of clonidine and the calcium antagonists used in MP-LM preparations from morphine tolerant animals were deliberately selected for their lack of significant effect on

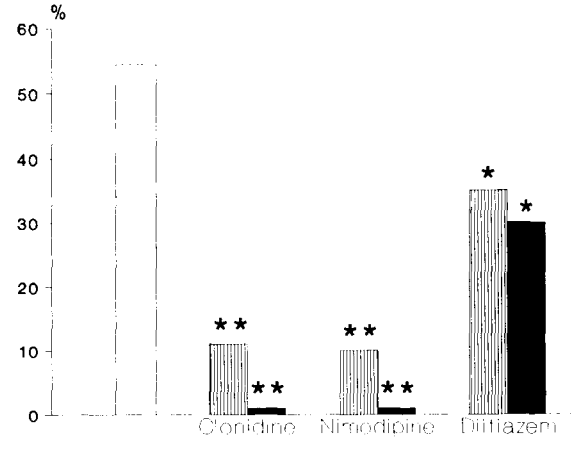

FIG. 1. Percentage of tissues exhibiting a contractile response to naloxone. Preparations were obtained from: dependent guinea-pigs, open columns; dependent guinea-pigs treated, 1 h before killing, with clonidine (75 $\mu g\ kg^{-1}$), nimodipine (5 mg kg^{-1}) or diltiazem (10 mg kg^{-1}), hatched columns; and dependent guinea-pigs whose MP-LM strips were incubated over 15 min with clonidine (10 nM), nimodipine (25 nM) or diltiazem (100 nM), solid columns. Each column is the mean % value from at least 8 preparations. * $P < 0.01$, ** $P < 0.001$ compared with untreated dependent group.

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contractile responses to electrical stimulation. When tissues were electrically stimulated over 15 min, the decrease of the height of the contractile response expressed as % was, in control preparations, $8{\cdot}4 \pm 1{\cdot}8$ (n = 7), in the presence of clonidine (10 nM), $21{\cdot}9 \pm 5{\cdot}3$ (n = 7), in the presence of nimodipine (25 nM), $8{\cdot}2 \pm 2{\cdot}8$ (n = 9), and in the presence of diltiazem (100 nM) $9 \pm 3{\cdot}5$ (n = 9).

These findings provide evidence to support the antiwithdrawal peripheral effects that have been suggested previously (Franz et al 1982; Baeyens et al 1987). All the drugs studied are able to inhibit the naloxone induced withdrawal sign, even though this decrease was associated with different mechanisms: clonidine activates α_2-presynaptic receptors and nimodipine and diltiazem reduce calcium entry to the smooth muscle by blocking the potential-operated calcium channels. Therefore, our results suggest that the effectiveness of these drugs in preventing the morphine withdrawal symptoms could be attributed at least in part, to peripheral mechanisms.

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