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Differential influence of D_1 and D_2 dopamine receptors on acute opiate withdrawal in guinea-pig isolated ileum

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1 The effects exerted by D_1 and D_2 dopamine agonists and antagonists on the acute opiate withdrawal induced by μ- and κ-receptor agonists were investigated *in vitro*.

2 Following a 4 min *in vitro* exposure to morphine (moderately selective μ-agonist), [D-Ala^2, Me-Phe^4, Gly-ol^5]enkephalin (DAMGO, highly selective μ-agonist) or U-50488H (highly selective κ-agonist) the guinea-pig isolated ileum exhibited a strong contracture after the addition of naloxone.

3 The non-selective dopamine receptor antagonist haloperidol when added before or after the opioid agonists, was able dose-dependently to prevent or to reverse the naloxone-induced contracture after exposure to μ- (morphine and DAMGO) and κ- (U-50488H) opioid agonists. The non-selective dopamine receptor agonist, apomorphine, was able to exert the same effects only at the highest concentration used.

4 The selective D_2 dopamine receptor antagonist, sulpiride, was also able to reduce dose-dependently both μ- and κ-opioid withdrawal, whereas the D_1-receptor selective antagonist SCH 23390 did not affect either μ- or κ-opioid withdrawal.

5 Bromocriptine, a D_2 selective dopamine receptor agonist was able to increase significantly, and in a concentration-dependent manner, the naloxone-induced contracture by μ- and κ-opioid agonists, whereas SKF 38393, a D_1 selective dopamine receptor agonist, increased only the withdrawal after morphine or U50-488H.

6 Our data indicate that both D_1 and D_2 dopamine agonists and antagonists are able to influence opiate withdrawal *in vitro*, suggesting an important functional interaction between the dopaminergic system and opioid withdrawal at both the μ- and κ-receptor level.

7 Furthermore, the ability of sulpiride to block strongly opiate withdrawal when compared to SCH 23390, as well as the effect of bromocriptine to increase opiate withdrawal suggest that D_2 dopamine receptors may be primarily involved in the control of opiate withdrawal.

Keywords: Dependence; opioids; dopamine; guinea-pig ileum; dopamine receptor agonists; dopamine receptor antagonists

Introduction

Opioid receptors are involved in a variety of functions, such as pain, nerve cell excitability and epilepsy, immunomodulation, stress, tolerance and dependence. The opiate withdrawal syndrome by opioids is a well-known phenomenon and its cellular mechanisms have also been studied (North & Karras, 1978; Collier, 1980; Collier *et al*., 1981; Johnson & Fleming, 1989).

It has been shown that in the development of opiate dependence a major role is played by μ-opioid receptors (De Launder *et al*., 1984; Gmerek & Woods, 1985). Until a few years ago the involvement of δ- and κ-opioid receptors in the development of opiate dependence was not well documented, since agonists and antagonists specifically acting at the δ- and κ-types of receptors were not available. However, recent evidence indicates that both δ- and κ-opioid receptors, as well as the μ-opioid receptor, are involved in the development of opiate physical dependence both *in vivo* and *in vitro* (Gmerek *et al*., 1987; Valeri *et al*., 1990c; 1992; Abdelhamid *et al*., 1991).

Although several methods may be used to produce opiate dependence both *in vivo* and *in vitro* (Johnson & Fleming, 1989), the similarities between the enteric nervous system and the central nervous system have made possible the widespread use of isolated preparations of intestine for investigations of the cellular biology of neurones (Wood, 1987). Thus the guinea-pig isolated ileum has provided a simple model for the study not only of the acute effects of opioids, but also of the long-term effects of tolerance and dependence, as the responses obtained from this tissue share many features in common with those observed in the central nervous system (Kosterlitz & Waterfield, 1975; Schulz & Herz, 1976; Leslie *et al*., 1980; Collier *et al*., 1981; Szerb, 1982). Significant advances in understanding dependence phenomena have been obtained, as it has been demonstrated that a strong naloxone-induced contracture could be obtained not only from the ileum of opiate-treated animals but also from untreated animals after a brief *in vitro* exposure to opioids (Lujan & Rodriguez, 1981; Collier *et al*., 1981; Chal, 1983; 1986; Valeri *et al*., 1990a,c; Morrone *et al*., 1990; 1993), thus indicating that the cellular mechanisms of dependence may occur very rapidly following occupation of receptors and that these mechanisms operate within the myenteric plexus. The characteristics of dependence development and the precipitation of withdrawal by naloxone in the guinea-pig ileum are very similar to those of acute dependence in experimental animals and man (Kosersky *et al*., 1974; Eisenberg, 1982; Krystal & Redmond, 1983; Bickel *et al*., 1988; Valeri *et al*., 1989; 1990a).

Brain dopaminergic systems have been widely implicated in many of the pharmacological effects of opioids. Manipulations that alter the activity of dopamine in the central nervous system (CNS) frequently modify the effects of morphine and other opioid drugs (Buxbaum *et al*., 1973; Eidelberg & Erspamer, 1975; Zarrindast & Mochaddampour, 1989; Gupta *et al*., 1988; 1989). Although the action of dopamine agonists and antagonists on opiate withdrawal has been studied (Lal *et al*., 1971; Gianutsos *et al*., 1974; Hynes *et al*., 1978), the mechanisms underlying this interaction are still unclear. In recent years, compelling evidence has accumulated to allow classification of CNS dopamine receptors into two distinct subtypes designated D_1 and D_2 on the basis of biochemical and phar-

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macological criteria. The recent availability of selective agonists and antagonists for dopamine D_1 and D_2 receptors provides powerful tools that can be used to determine the roles of these receptor types in mediating some of the physiological and pharmacological effects of dopamine in the CNS.

The experiments described here were undertaken to provide insight into the role of specific dopamine receptor subtypes in mediating opioid withdrawal. Although it has been demonstrated that dopamine agonists exacerbate the opiate withdrawal syndrome, whereas antagonists such as haloperidol decrease the severity of the syndrome (Lal *et al*., 1971; Gianutsos *et al*., 1974; Hynes *et al*., 1978), there are no data available, to our knowledge, on the effect exerted by selective D_1 and D_2 dopamine agonists and antagonists on the acute opiate-dependence induced by opioid agonists. Therefore, the aim of the present study was to test whether selective dopamine agonists and antagonists are able to modify opiate withdrawal through the involvement of D_1 and/or D_2 dopamine receptors. Haloperidol was used as a non-selective dopamine receptor antagonist, and SCH 23390 and sulpiride as D_1- and D_2-receptor selective antagonists, respectively (Zarrindast & Moghaddampour, 1989); apomorphine was used as a non-selective dopamine receptor agonist, and SKF 28393 and bromocriptine as D_1- and D_2-selective dopamine receptor agonists, respectively (Zarrindast & Moghaddampour, 1989).

The effects of dopamine receptor agonists and antagonists were evaluated on opiate withdrawal induced by morphine (moderately selective μ-agonist), [D-Ala^2, Me-Phe^4, Gly-ol^5] enkephalin (DAMGO, highly selective μ-agonist) and U-50488H (highly selective κ-agonist) to test whether the possible interaction of dopamine on opioid withdrawal involves μ- and/or κ-opioid receptors.

Methods

Animals

Adult male guinea-pigs (200–250 g) purchased from Charles River, Italy were used in the experiments. Animal Care and use followed the directions of the Council of the European Communities (1986). The animals were housed in colony cages (4 guinea-pigs each) with free access to food and water; they were maintained in a climate- and light-controlled room (22 ± 1°C, 12/12 h dark/light cycle with light on at 0.7 h 00 min) at least 7 days before testing.

Preparation of guinea-pig isolated ileum

The animals were killed by CO_2 inhalation and bled. The terminal portion of the ileum (the 10 cm nearest the caecum was discarded), was kept in a Petri dish with Tyrode solution (g l^{-1}: NaCl 8.00, KCl 0.20, $CaCl_2$ 0.20, $MgCl_2.6H_2O$ 0.10, $NaH_2PO_4.2H_2O$ 0.05, $NaHCO_3$ 1.00 and glucose 1.00) for 30 min and then washed free of faecal matter. Two to four segments, 2–3 cm long, from the same animal were placed between platinum electrodes and connected to an 85/2/50 model M.A.R.B. Stimulator (Ditta M.A.R.B., Chiesina Uzzanese, Pistoia, Italy). A force-displacement transducer and unirecord model polygraph was used for measurement of isotonic contractions (Ugo Basile, Milano, Italy). A resting tension of 0.5 g was applied. The baths were maintained at 37°C and continuously bubbled with a mixture of 95% O_2 and 5% CO_2.

Acute opiate dependence on guinea-pig isolated ileum

The experimental procedure was that described previously (Schulz & Herz, 1976; Valeri *et al*., 1990a) with modifications (Capasso *et al*., 1996). The preparations of ileum were allowed to equilibrate for 40–60 min without washing and the response to acetylcholine (ACh 10^{-6} M) was determined three times so that responses could be expressed as a percentage of the ACh maximum. A reproducible acute opiate dependence was obtained by performing the following experimental procedure. A typical tracing of contracture responses of the ileum to repeated challenges with opiate and naloxone is shown in Figure 1.

After three similar ACh responses were obtained, the preparation was electrically stimulated for 10–20 min (0.5 ms pulse delivered transmurally, at a frequency of 0.1 Hz at supramaximal voltage 25 V). Before the addition of the opioid agonist (morphine, DAMGO or U-50488H) to the bath the electrical stimulation was switched off and under these conditions, 4 min after the first contact with the opioid agonist, exposure to naloxone induced a strong contracture (about 60% of the ACh maximum). Following washout the responsiveness of the ileum after withdrawal was tested by the addition of ACh (Figure 1a) and after a 30 min resting period of electrical stimulation, the 4 min exposure of the ileum to the opioid and then naloxone elicited a reproducible contractile response. Following washout the response to ACh was obtained (Figure 1b), and after another 30 min resting period under stimulation, the ileum responded again to the opioid agonist and naloxone

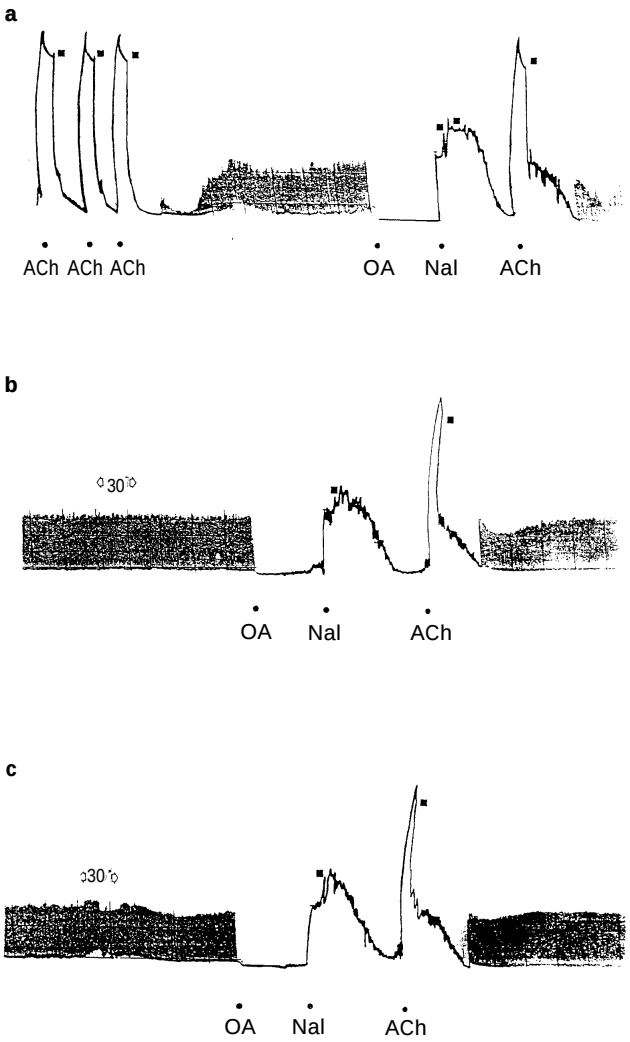

Figure 1 Typical tracing of opioid withdrawal on guinea-pig ileum. (a) Three similar responses to acetylcholine (ACh) were obtained, and after the period of electrical stimulation, opioid agonist (OA) was added followed after a 4 min contact period by naloxone (Nal) which induces contaction (1° opioid withdrawal). After washout (■), the addition of acetylcholine was repeated. (b) After 30 min resting period under electrical stimulation, a further 4 min exposure of the ileum to OA and naloxone elicited a reproducible withdrawal response (2° opioid withdrawal). (c) After another 30 min resting period under electrical stimulation, the ileum responded again to the OA and naloxone with the same intensity (3° opioid withdrawal).

with the same intensity (Figure 1c). In our experiments, to avoid the possible development of tolerance to repeated exposure to the opioid, each preparation was submitted to only three challenges with the opioid agonist and naloxone. Naloxone by itself did not produce effects on 'naive' preparations or those washed out after contact with the opioid agonist.

Experimental procedure

The administration of dopamine agonists and antagonists was performed according the following schedule: (a) 3 ACh responses; (b) electrical stimulation (10–20 min); (c) opiate agonists administrated in the absence of electrical stimulation (4 min) and addition of naloxone with subsequent contraction (1° opioid withdrawal); (d) washout and ACh response; (e) electrical stimulation (30 min); (f) dopamine agonists or antagonists (10^{-6}, 5×10^{-5} and 10^{-5} M) without electrical stimulation, injected 10 min before or after the opioid agonist (morphine, DAMGO or U-50488H), followed by naloxone (2° opioid withdrawal); (g) washout and ACh response; (h) electrical stimulation (30 min); (i) final control opiate withdrawal (3° opioid withdrawal).

In these experiments, dopamine receptor agonists or antagonists were administered 10 min before or after the administration of the opioid agonist. Since during exposure to dopamine agonist or antagonist the duration of the contact period of the opioid agonist was 10 min, to avoid a possible influence of the contact period we performed a series of preliminary experiments to verify whether a contact period longer than 4 min might affect the naloxone contracture. No differences were observed when the period exposure to the opioid agonist was 4 or 10 min.

In our experimental conditions, after a series of preliminary experiments to induce a strong contracture, each opioid agonist and naloxone were administered at the following concentrations: morphine (10^{-5} M) + naloxone (10^{-5} M; DAMGO (10^{-6} M) + naloxone (10^{-6} M); U-50488H (10^{-8} M) + naloxone (10^{-5} M).

Each experiment was performed on 6 to 9 preparations from different animals.

Drugs

All drugs were purchased from the Sigma Chemical Co (St. Louis, U.S.A.) with the exception of morphine HCl from Carlo Erba (Milan, Italy), U-50488H (*trans*-(±)-3,4-dichloro-N-methyl-N-[2-(l-pyrrolidinyl)-cyclohexyl]-benzeneacetamide) from the Upjohn Co. (Kalamazoo, MICH, U.S.A.), and SCH 2339 ((R)-(+)-8-chloro-2,3,4,5, tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol-hemimaliate) and SKF 38393 (R (+)-1-phenyl-2,3,4,5-tetrahydro (1H)-3-benzazepin 7,8-diol) from RBI (Natick, U.S.A.).

Parameter evaluation

Four parameters were measured:

(1) Naloxone contracture The size of the contracture produced by the naloxone challenge was expressed as a fraction of the maximum contraction obtained with the subsequent addition of ACh in the same piece of tissue according to a modification of the method of Collier *et al.* (1981): (Response to naloxone)/(Maximum response to ACh) × 100 = tension ratio.

(2) ACh responses before and after treatment Any reduction or increase of the ACh responses in the post-drug period was expressed as a percentage of the ACh response in the pre-drug period.

(3) Electrical stimulation contraction before and after treatment Reduction or increase of the electrical stimulation contraction in the post-drug period was expressed as a percentage of the electrical stimulation contraction in the pre-drug period.

(4) Naloxone contraction before and after treatment Reduction or increase of the naloxone contraction in the post-drug period was expressed as a percentage of the naloxone-induced contraction in the pre-drug period.

Statistical analysis

Results were tested for statistical significance by use of Student's *t* test for paired data when results before and after treatments on the sample preparation were compared.

Results

Effect of dopamine antagonists haloperidol, SCH 23390 and sulpiride on withdrawal responses to morphine, DAMGO and U-50488H

The addition of haloperidol (10^{-6}, 5×10^{-6} and 10^{-5} M) 10 min before or after morphine, DAMGO or U-50488H produced a concentration-dependent reduction of the opiate withdrawal induced by the μ- and κ-agonists (Figure 2a). The selective D_1 dopamine receptor antagonist SCH 23390 (10^{-6}, 5×10^{-5} and 10^{-5} M) did not affect the opiate withdrawal induced by the μ- and κ-agonists (Figure 2b) whereas the selective D_2 dopamine receptor antagonist sulpiride at the same concentrations was able to reduce significantly and dose-dependently the μ- and κ-opioid withdrawal (Figure 2c). The same effects were obtained when the drugs were injected 10 min before the opioid agonists (Data not shown).

After washout, the response to ACh was not affected by the dopamine antagonists whereas the final opiate withdrawal responses were still reduced.

The effect of dopamine agonists apomorphine, SKF 38393 and bromocriptine on withdrawal responses to morphine, DAMGO and U-50488H

The addition of apomorphine (10^{-6}, 5×10^{-6} and 10^{-5} M) 10 min before or after morphine, DAMGO or U50-488H produced a significant reduction of μ- or κ-opioid withdrawal only at the highest concentration used (Figure 3a).

The selective D_2 dopamine receptor agonist bromocriptine was able to increase significantly and dose-dependently both μ- and κ-opioid withdrawal (Figure 3b), whereas the D_1 agonist SKF 38393 increased the withdrawal response after morphine and U-50488H, but not that with the selective μ-agonist DAMGO (Figure 3c). The same results were obtained when the drugs were injected 10 min before the opioid agonists (data not shown).

After washout, the ACh response was not affected by the treatments with dopamine receptor agonists, whereas the final opioid withdrawal responses were still increased.

Discussion

The present study indicates that both dopamine receptor agonists and antagonists, added before or after opioid agonists, induce significant effects on opiate withdrawal *in vitro* thus confirming an important involvement of dopamine receptors in the control of opioid withdrawal (Lal *et al.*, 1971; Gianutsos *et al.*, 1974; Hynes *et al.*, 1978).

Under our experimental conditions, the non-selective dopamine receptor antagonist haloperidol was able both to prevent and reverse the acute withdrawal induced by naloxone after treatment with two μ-agonists morphine and DAMGO, and the κ-agonist U-50488H. The reduction by haloperidol of the opioid withdrawal contracture was concentration-dependent consistent with an action mediated by a dopamine receptor.

Dopamine mediates its effects through at least two dopamine receptor types, D_1 and D_2 (Stoof & Kebabian, 1984) and

in the present study the possible involvement of a specific dopamine receptor in mediating opiate withdrawal was also considered. The experiments performed with the selective D_1 and D_2 dopamine receptor agonists and antagonists showed that it is the D_2 dopamine receptor subtype that is mainly involved in the control of opiate withdrawal. Thus the selective D_2 dopamine receptor antagonist sulpiride was able both to prevent and reverse acute withdrawal induced by naloxone after treatment with the μ- and κ-agonists, whereas SCH 23390, a D_1 dopamine receptor antagonist, did not have any effect on either μ- or κ-mediated withdrawal.

Interestingly, our results with the non-selective dopamine receptor agonist apomorphine, showed a reduction at the highest concentration used (10^{-5} M) of μ- and κ-opioid withdrawal. A similar concentration-related interaction was observed in experiments performed by Gupta *et al.* (1989) with apomorphine on morphine analgesia. It was suggested that apomoprhine, depending on the doses used, exibited a differential activation of dopamine receptors, with high doses of apomorphine stimulating postynaptic dopamine receptors (D_1). Therefore, the results of the present experiments could be explained on the basis of actions on pre- and post-synaptic

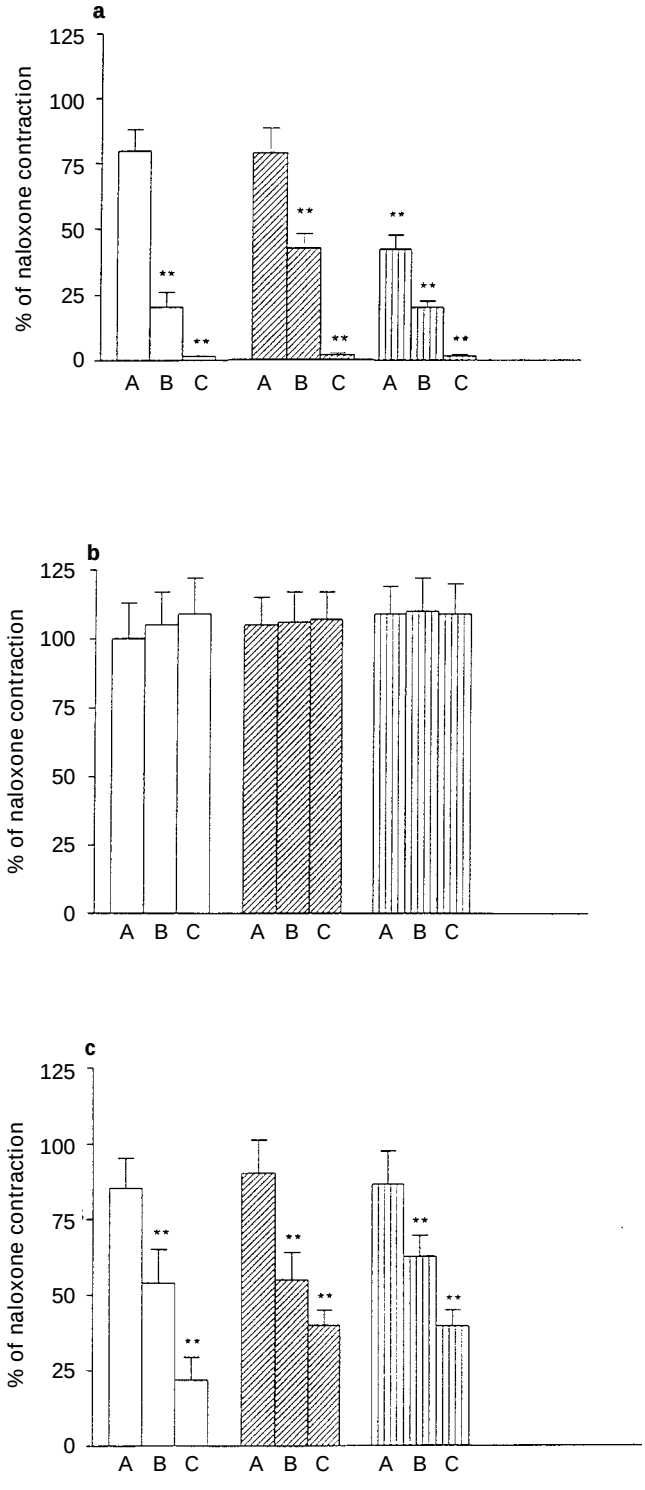

Figure 2 Concentration-related effect of haloperidol (a), SCH 23390 (b) and sulpiride (c) on morphine (open columns), DAMGO (hatched columns) and U-50488H (vertical striped columns) withdrawal; (A)

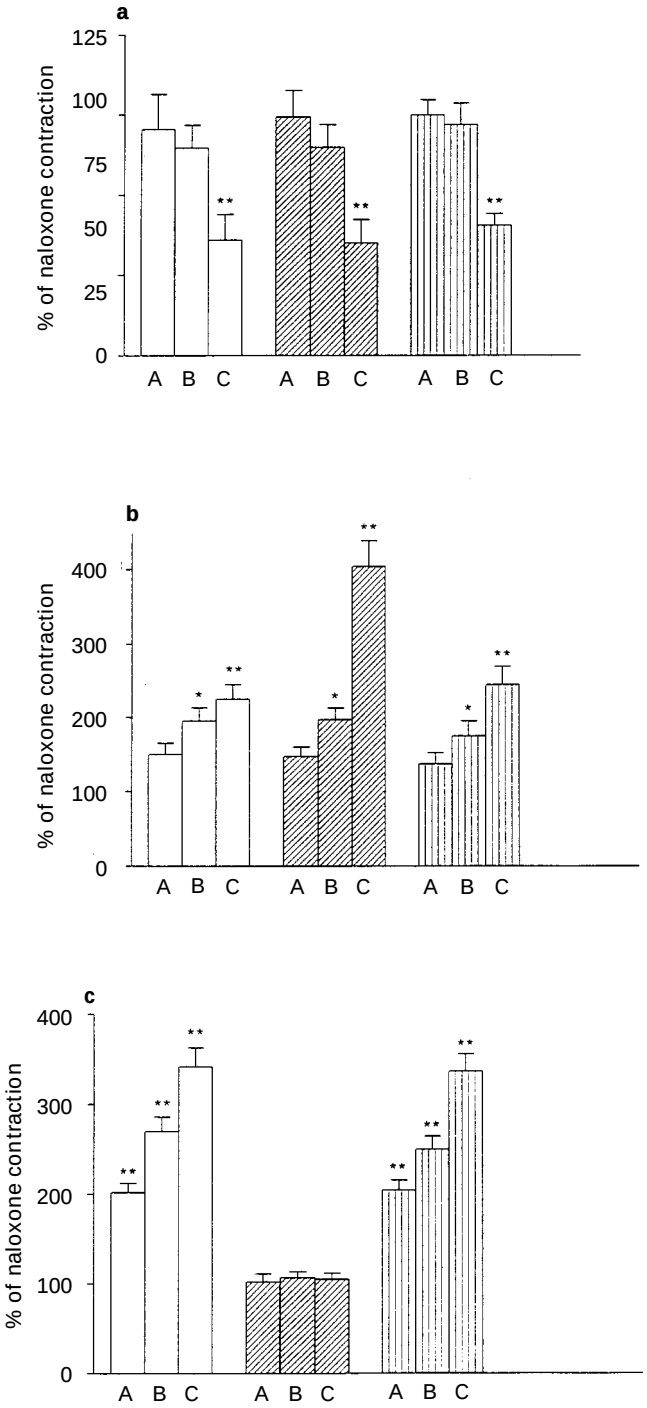

Figure 3 Concentration-related effect of apomorphine (a), bromocriptine (b) and SKF 38393 (c) on morphine (open columns), DAMGO (hatched columns) and U-50488H (vertical striped columns) withdrawal; (A) 1×10^{-6} M, (B) 5×10^{-6} M, (C) 1×10^{-5} M, $^{*}P<0.05$; $^{**}P<0.01$.

receptors, as previously described (Gupta *et al*., 1989), suggesting that the inhibition induced by haloperidol is related to presynaptic receptor (D_2) block whereas the inhibition by apomorphine may be related to postsynaptic receptor (D_1) stimulation.

Our results further confirm the opposite influences of D_1 and D_2 dopamine receptors on opioid effects (Di Chiara *et al*., 1976; 1977; Setler *et al*., 1978; Gianutsos & Moore, 1980; Costall *et al*., 1980; Robertson *et al*., 1981; Stoof & Kebabian, 1982; 1984; Kendler *et al*., 1982; Hyttel, 1984), since SKF 38393 and bromocriptine, D_1 and D_2 dopamine receptor agonists, respectively, significantly increase opioid withdrawal. However, it is of interest to observe that SKF 38393 was able to increase only morphine and U50-488H withdrawal without altering DAMGO dependence, indicating that the selective D_1 receptor agonist is able only to influence κ-mediated opiate withdrawal. This may be related to the different intracellular biochemical mechanism mediating the inhibitory actions of opioids on the myenteric neurones since μ-opioid agonists increase potassium conductance, whereas κ-agonists reduce calcium conductance (North, 1986). However, although it seems that the effect induced by κ-opioid agonist withdrawal is mainly due to the excitation of the cholinergic neurone, as with μ-agonists, it is unknown whether these two opioid agonists activate the same neurones, and whether the sequence of biochemical and neuronal events leading to the development of dependence and its symptoms is different for the two agonists (Valeri *et al*., 1990b).

Given the above experiments, it is postulated that D_2 dopamine receptors are important for their involvement in the control of opiate withdrawal, since D_2 dopamine receptor agonists increased while D_2 dopamine receptor antagonists reduced the opiate withdrawal.

Regarding the possible mechanism by which D_2 agonists and antagonists control opiate withdrawal, it is hypothesized that the effects observed are related to alterations in the levels of adenosine 3′ : 5′-cyclic monophosphate (cyclic AMP). Cyclic AMP has frequently been implicated as an intracellular messenger for the receptor-mediated actions of opioids. Biochemical observations has indicated that opioids inhibit adenylate cyclase activity and decrease the level of cyclic AMP (Collier & Roy, 1974; Collier, 1980; Schramm & Selinger, 1984; Worley *et al*., 1987; Neher, 1988). More recently, it has been shown that adenylate cyclase activity is also present in guinea-pig myenteric neurones and that morphine decreases the activity of the enzyme (Jeitner & Costa, 1989). Opioid withdrawal produces adenylate cyclase hyperactivity associated with an intracellular increase of cyclic AMP (Ho *et al*., 1973a,b).

D_1 and D_2 dopamine receptors are coupled to adenylate cyclase and stimulation of D_1 receptors causes an increased production of cyclic AMP, whereas stimulation of D_2 receptors causes a decrease of cyclic AMP (Kebabian & Calne, 1979; Stoof & Kebabian, 1981; 1984; Onali *et al*., 1984). However, the ability of haloperidol, sulpiride and apomorphine to reduce opioid withdrawal, with the ability of D_1 and D_2 agonists to potentiate opioid withdrawal are difficult to relate to changes in cyclic AMP production.

One possibility would be that other neurotransmitters are involved in the withdrawal contracture. It has been shown that a large proportion of the contracture is due to acetylcholine release since it is blocked by atropine or hyoscine (Tsou *et al*., 1982; Chal, 1983). In our experiments we exclude the possibility of a direct action of dopamine receptor agonists or antagonists on postsynaptic acetylcholine receptors, since responses to exogenous acetylcholine were not modified in the guinea-pig ileum after dopamine receptor agonist or antagonist treatment.

Finally, whatever the mechanism may be, our data indicated that the dopaminergic system exerts an important control on the opioid withdrawal phenomenon. The powerful actions of D_2 agonists and antagonists on the opiate withdrawal response suggest that it is the dopamine D_2 receptor that is mainly involved in the control of physical dependence.

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