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Effects of Opioid and α_2-Adrenoceptor Agonists on the Isolated Ileum of Morphine-dependent Guinea-pigs During Withdrawal and After Clonidine Treatment

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Abstract—The present study was undertaken to investigate the effect of clonidine administration to opiate-dependent guinea-pigs after morphine withdrawal on subsequent twitch responses of the longitudinal muscle-myenteric plexus preparations to electrical field stimulation. The results indicate that clonidine, administered immediately after morphine removal, causes tolerance to the inhibition exerted by opioid and α_2-adrenoceptor agonists on the electrically-evoked twitches. Such a finding suggests that the mechanism of action of clonidine involves not only its well-known effects on locus coeruleus neurons but also that it has specific actions on the myenteric plexus. This work shows the existence of interactions between opioid and α_2-adrenoceptor on the cholinergic neurons present in the isolated ileum.

Clonidine, an α_2-adrenoceptor agonist and antihypertensive agent, reduces many of the manifestations of opiate withdrawal in animals and man. Clonidine has been employed in the treatment of heroin and methadone withdrawal symptoms (Devenyi et al 1982; Washton & Resnick 1982). In rats, it suppresses some symptoms of the morphine withdrawal syndrome such as body shakes (Fielding et al 1978) and body weight loss (Sparber & Meyer 1978).

Several investigators have proposed that the ability of clonidine to attenuate the opiate withdrawal is mediated by α_2-adrenoceptors located on the cell bodies of noradrenergic neurons in the locus coeruleus, thereby inhibiting the increased noradrenergic turnover during withdrawal (Gold et al 1981). This finding seems to indicate that inhibitory α_2-adrenoceptors could be directly involved in the development of the opiate abstinence syndrome.

It has been suggested that the prejunctional α_2-adrenoceptors present on cholinergic nerve terminals in the guinea-pig ileum are of a similar type as those found prejunctionally on sympathetic neurones (Drew 1978).

In addition, the guinea-pig isolated ileum is a simpler model of opiate dependence since it shows two of the basic characteristics of opiate dependence in the whole animal: (a) the response of an isolated ileum obtained from a chronic morphine-treated guinea-pig to naloxone is confined to a single response, i.e. the withdrawal contracture of the longitudinal muscle (Schulz & Herz 1976); (b) this effect is accompanied by a high degree of tolerance to the acute action of opiate on the ileum (Schulz et al 1981; Wuster et al 1981).

The present study was designed to investigate two questions. First, since chronic morphine administration produces tolerance in guinea-pig isolated ileum, to what extent is the tolerance limited to this specific opioid receptor population (selective tolerance) leaving the sensitivity of other types unaffected? Second, since clonidine is able to substitute for morphine in morphine-dependent rats, can in-vivo administration modify the adaptative changes evoked in ileum preparations after morphine deprivation?

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Materials and Methods

Male guinea-pigs, 300–350 g, were killed by a blow on the neck and the ileum was rapidly excised. The longitudinal muscle myenteric plexus (LM-MP) was prepared as described by Kosterlitz et al (1970). A strip (2 cm) was set up in a 40 mL organ bath in Krebs-bicarbonate buffer (composition in mM: NaCl 118, KCl 4·75, $CaCl_2$ 2·54, $MgSO_4$ 1·20, KH_2PO_4 1·19, $NaHCO_3$ 25·0, glucose 11) oxygenated with 95% O_2–5% CO_2 at 37°C as described by Goldstein & Schulz (1973). The tissues were suspended under a resting tension of 1 g and were allowed to equilibrate for 60 min. Contractions were induced by field stimulation of 0·3 Hz, 0·2 ms pulse duration and supramaximal voltage. Responses were recorded via a force displacement transducer coupled to a polygraph recorder.

The drug effects evoked were expressed as percentage inhibition of the electrically-induced twitches.

Assessment of opiate tolerance

Guinea-pigs were rendered tolerant in 3 days by the subcutaneous implantation of 4 morphine pellets (each containing 75 mg morphine base). Tolerance and cross-tolerance were measured in the organ bath by comparing the effect of morphine and clonidine on electrically-evoked contractions of ileum preparations from guinea-pigs treated and untreated with morphine.

The opiate-like inhibition of twitch contractions of the LM-MP elicited by high frequency (tetanic) electrical stimulation as described by Puig et al (1977) was also studied.

Assessment of opiate dependence

Dependence was measured in electrically-stimulated preparations in terms of the inhibition induced either by application of morphine or clonidine or by tetanic electrical stimulation. Withdrawal syndrome was induced by removing pellet residues on the 3rd day after implantation and the

animals were maintained for up to three days under a 12 h light/dark cycle with free access to food and water. Implantation and removal were carried out under light ether anaesthesia. Ileal strips were obtained on either the first, second or third day after pellet removal.

In order to study the effect of clonidine on the withdrawal syndrome, clonidine (0·5 mg kg^{-1}) was injected intraperitoneally twice daily immediately after morphine removal. The first injection was administered at the same time as morphine removal and the last 1 h before killing. Animals were killed 24, 48 and 72 h after morphine removal as described above.

Clonidine was also injected twice daily over three days in non-opiate treated guinea-pigs. These animals were killed 1 h after the final injection.

Drugs and statistics

Morphine base and morphine hydrochloride were from Alcaliber, S.A. Spain and clonidine hydrochloride was from Boehringer Ingelheim, S.A. Barcelona, Spain.

Comparisons between experimental groups were made using Student's *t*-test. Data are expressed as mean ± s.e.m.

Results

Morphine (0·2 μM) or clonidine (0·2 μM) administration to LM-MP preparations from control animals induced an inhibition of the twitch contraction, these effects being prevented and reversed, in a statistically significant way, by naloxone (0·1 μM, n = 8) or yohimbine (1 μM, n = 8), respectively. Naloxone (0·1 μM) was also able to prevent and reverse the post-tetanic inhibition elicited by tetanic electrical stimulation (data not shown).

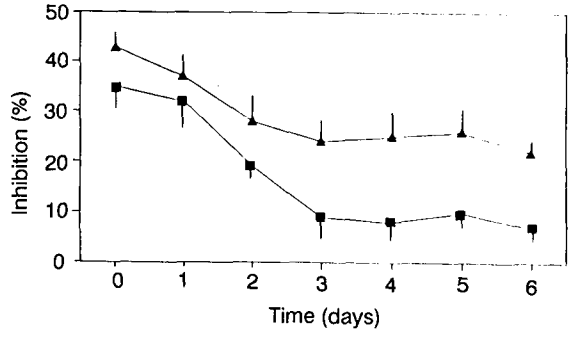

FIG. 1. Effects of morphine (0·2 μM, ■) and clonidine (0·2 μM, ▲) on the longitudinal muscle-myenteric plexus from chronic morphine guinea-pigs. Morphine treatment was carried out by subcutaneous implantation of 4 morphine pellets (each containing 75 mg morphine base). Guinea-pigs were killed 1–6 days after pellet implantation. Each point reflects the mean inhibition (%) of the electrically-evoked twitch on 8 isolated tissues of different animals. Vertical bars are s.e.m.

Fig. 1 shows that the size of the inhibitory response to either morphine (0·2 μM) or clonidine (0·2 μM) of LM-MP preparations isolated from guinea-pigs during exposure to morphine was gradually decreased until the third day, after which a plateau was reached.

After removal of the pellets on the third day, tolerance to morphine (0·2 μM) and clonidine (0·2 μM) decreased gradually as indicated by the increase of the twitch inhibition induced by the same dose of these compounds with respect to data obtained on preparations from tolerant animals (Table 1). The inhibition of the twitch reached a maximum 2 days after pellet removal (Table 1), and was even greater than that exerted by the same concentration of morphine (35 ± 3·3, $P < 0.01$) and clonidine (43 ± 3·4, $P < 0.05$) in LM-MP preparations from control animals. A normal sensitivity restored by the third day after pellet removal was also observed (Table 1).

Tetanic electrical stimulation of the LM-MP preparations from guinea-pigs treated with morphine over 3 days (Table 1) produced an inhibition of twitch contraction significantly lower than that of control animal preparations (85 ± 2·5, $P < 0.001$). This effect was completely reversible and after abrupt morphine withdrawal (Table 1), post-tetanic inhibition was similar to that registered in control animals.

Clonidine treatment after abrupt morphine withdrawal induced, 24 and 48 h after pellet removal, a response to morphine (0·2 μM), clonidine (0·2 μM) and tetanic electrical stimulation that was similar to that seen in tolerant animals (Table 2).

When clonidine was administered over the 72 h after morphine withdrawal, the inhibitory effect induced by morphine (0·2 μM) and opiate-like material was greater than in non-clonidine treated animals (Table 2). However, the effect of clonidine (0·2 μM) was not modified compared with the corresponding control animals (Table 2).

On the other hand, clonidine treatment administered over 72 h to opiate-untreated guinea-pigs also significantly increased the inhibition induced by morphine (0·2 μM) (35 ± 3·3 to 59 ± 4·7, $P < 0.001$), while the inhibition elicited by clonidine (0·2 μM) and opiate-like material was not modified (43 ± 3·4 to 49 ± 7·4 and 85 ± 2·5 to 93 ± 1·2, respectively).

Table 1. Effect of morphine (0·2 μM), clonidine (0·2 μM) and opiate-like material on longitudinal muscle-myenteric plexus from chronic morphine guinea-pigs and after morphine removal.

		Time after withdrawal (h)				
	Chronic	12	18	24	48	72
Morphine	9 ± 3·1	18 ± 2·4*	22 ± 3·4**	35 ± 5·1***	50 ± 3·6***	29 ± 3·1***
Clonidine	24 ± 3·4	26 ± 4·6	29 ± 4·1	42 ± 5·3**	55 ± 5·3***	38 ± 3·1**
Opiate-like material	29 ± 4·8	48 ± 4·7**	70 ± 3·4***	81 ± 1·5***	86 ± 2·1***	85 ± 2·3***

Morphine withdrawal was carried out by pellet removal on the 3rd day, the animals being killed 12, 18, 24, 48 and 72 h later. Values are expressed as percentage of inhibition of the electrically-evoked twitch (mean ± s.e.m., n = 8). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (Student's *t*-test).

Table 2. Effect of clonidine treatment on the inhibition induced by morphine (0·2 μM), clonidine (0·2 μM) and opiate-like material in longitudinal muscle-myenteric plexus from morphine abstinent guinea-pigs.

		Time after morphine withdrawal (h)		
	Treatment	24	48	72
Morphine	Withdrawal	35 ± 5·1	50 ± 3·6	29 ± 3·1
	Withdrawal + clonidine	2 ± 1·2***	8 ± 2·0***	54 ± 4·4***
Clonidine	Withdrawal	42 ± 5·3	55 ± 5·3	38 ± 3·1
	Withdrawal + clonidine	15 ± 2·7***	14 ± 1·9***	44 ± 5·5
Opiate-like material	Withdrawal	81 ± 1·5	86 ± 2·1	85 ± 2·3
	Withdrawal + clonidine	33 ± 5·7***	45 ± 5·9***	91 ± 1·7*

Clonidine (0·5 mg kg^{-1}) was injected intraperitoneally twice daily immediately after morphine removal. Guinea-pigs were killed 24, 48 and 72 h later. Values are expressed as percentage of inhibition of the electrically-evoked twitch (mean ± s.e.m. n = 8). * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$ (Student's t-test).

Discussion

Our experiments confirm that morphine and clonidine inhibit the electrically-evoked contractions of the LM-MP preparations from guinea-pigs by acting on opioid and α_2-adrenoceptors, since naloxone and yohimbine respectively are able to prevent and reverse such effects. This fact results from the ability of morphine and clonidine to inhibit the release of acetylcholine in this tissue (Paton 1957; Gillan et al 1979). High frequency electrical stimulation of the LM-MP preparation resulted in the post-tetanic inhibition of subsequent neurogenic twitches. It has been suggested that this phenomenon is mainly due to the release of opiate-like material from the myenteric plexus of the guinea-pig ileum (Puig et al 1977), metenkephalin and dynorphin being the most likely candidates as the endogenous mediators of this effect (Horacek & Kadlec 1984). Pretreatment of the guinea-pigs with morphine resulted in a reduction in the sensitivity of isolated ileum preparations to morphine, clonidine and endogenous opioids. Thus, it would appear that in-vivo chronic activation of a specific opiate receptor type (μ-receptors) causes tolerance not only on these specific receptors, but also on other receptor types (α_2-adrenoceptors). Therefore, the opioid-induced desensitization is non-specific with regard to other compounds which exert their inhibitory effects via α_2-adrenoceptors. These results contrast with studies demonstrating in the guinea-pig isolated ileum that tolerance may develop to only one type of receptor (selective tolerance) (Schulz et al 1981). However, more recently other authors (Bentley et al 1983; Post et al 1988) have reported the existence of cross-tolerance between opioid and α_2-adrenoceptors in the peripheral nervous system. Our findings are consistent with those reported by these latter authors.

Theoretically, our findings in the guinea-pig ileum require a common system with which opiate and α_2-adrenoceptors interact. One possibility is that receptors with a presynaptic location may share a common second messenger signal, such as cAMP resulting from adenylate cyclase activity. Therefore, an alteration in the intracellular effector linked to the μ-receptor could result in cross-tolerance to other agonists if the latter acted via the same intracellular effector process. The α_2-adrenoceptor agonist clonidine has similar effects on myenteric S neurons as does morphine. Both produce membrane hyperpolarization associated with an increase in potassium conductance (Suprenant & North 1985). If the μ-receptor and α_2-adrenoceptor were linked to the same potassium channels via a common intracellular effector, an alteration in the common pathway could result in tolerance to both agents. In this sense, it has been described that in the rat locus coeruleus, the levels of adenylate cyclase activity increase during chronic administration of either morphine (Duman et al 1988) or clonidine (Nestler et al 1989); similar effects of both drugs on adenylate cyclase had been reported previously in cultured neuroblastoma glioma cells (Sharma et al 1975; Sabol & Nirenberg 1979).

On the other hand, pharmacological alterations observed during morphine tolerance in the guinea-pig myenteric plexus are completely reversible. Within 3 days, following abrupt withdrawal, sensitivities to morphine, clonidine and endogenous opioid peptides returned to normal levels. Thus, these findings support the view that opiate tolerance can disappear rapidly and completely (Schulz et al 1974). In addition, 48 h after morphine removal, a supersensitivity of opioid and α_2-adrenoceptors takes place, as shown by the major inhibitory effect exerted by morphine and clonidine. It has been proposed that supersensitivity results from an adaptative increase in the number of receptors for morphine (Rogers & El-Fakahany 1986) and clonidine (Ulibarri et al 1987; Smith et al 1989). However, the non-specific supersensitivity suggests that the underlying mechanism involves a more fundamental change in neuronal excitability, such as a membrane depolarization, rather than changes in different receptors (Johnson & Fleming 1989).

Clonidine administered to guinea-pigs at 24 and 48 h after morphine removal, not only prevented the responses described above, but also caused the appearance of tolerance to morphine, clonidine and endogenous opioid peptides in the tissue studied. These findings indicate that the effects induced on the guinea-pig isolated ileum by morphine withdrawal, can be suppressed, at least temporally, by clonidine administration, and suggest that clonidine could substitute for morphine in opiate-dependent guinea-pigs.

Clonidine has been demonstrated to be an effective non-opiate treatment for opiate detoxification in man (Gold et al 1978), since it shows considerable parallels with the action of opiate receptor agonists in modifying the excitability of the locus coeruleus neurons by acting on different receptors. The firing of locus coeruleus neurons decrease after acute admin-

istration of either morphine or clonidine (North et al 1987), returns to control values after chronic treatment, and increases above control levels following abrupt removal of drug treatment in-vivo (Engberg et al 1982; Christie et al 1987). Our results suggest that the interactions between opioid and α_2-adrenoceptor agonists are probably not restricted to those receptor sites within the central nervous system but also extend to the peripheral nervous system.

On the other hand, clonidine administered to opiate-untreated guinea-pigs increased significantly the morphine-induced inhibition in the organ bath. Similar findings have also been described by other authors. Thus clonidine potentiates the effects of intrathecal morphine or results in a synergism giving rise to enhanced analgesia (Hylden & Wilcox 1983; Sullivan et al 1987). Clinicians have reported the use of spinal clonidine in conjunction with morphine to potentiate analgesia (Coombs et al 1985; Tamsen & Gordh 1984).

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