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European Journal of Pharmacology 376 (1999) 265–271

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Cross-tolerance and convergent dependence between morphine and cannabimimetic agent WIN 55,212-2 in the guinea-pig ileum myenteric plexus

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Received 7 January 1999; received in revised form 18 May 1999; accepted 21 May 1999

Abstract

The cross-tolerance and convergent dependence between morphine and the cannabimimetic agent *R*(+)-[2,3-dihydro-5-methyl-3[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl) methanone mesylate (WIN 55,212-2) were assessed in vitro on guinea-pig ileum. To induce tolerance and dependence the myenteric plexus-longitudinal muscle was incubated at 37°C for 5 h with a fixed concentration representing the IC_{50} for each compound. Myenteric plexus-longitudinal muscle exposed to WIN 55,212-2 (5×10^{-8} M) was less sensitive to its inhibitory effect on electrically evoked contractions than naive myenteric plexus-longitudinal muscle. The exposure to cannabinoid induced a parallel rightward shift in the lower part of the concentration–response curve of WIN 55,212-2 and a marked reduction in the maximal inhibitory effect of the drug. Myenteric plexus-longitudinal muscle tolerant to WIN 55,212-2 was subsensitive to the inhibitory effect of morphine on the twitch response. The cross-tolerance between WIN 55,212-2 and morphine was bidirectional. In fact, after 5 h the morphine (10^{-7} M)-incubated myenteric plexus-longitudinal muscle was less sensitive to the inhibitory effect of WIN 55,212-2. The tissue tolerant to morphine or WIN 55,212-2 was tested for the presence of physical dependence. Naloxone (10^{-5} M) produced a typical withdrawal contracture in morphine-tolerant myenteric plexus-longitudinal muscle which could be reduced by a 15-min pretreatment with WIN 55,212-2 (5×10^{-8} M). In contrast, SR141716 (10^{-6} M) [*N*-(piperidino)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide], a concentration which fully antagonized the inhibitory effect of WIN 55,212-2 (10^{-7} M) in control preparations, did not produce significant contracture in WIN 55,212-2-tolerant myenteric plexus-longitudinal muscle. The mechanisms underlying the cross-tolerance and convergent dependence remain to be ascertained.

Keywords: WIN 55,212-2; Morphine; Ileum; (Guinea-pig); Cross-tolerance; Convergent dependence

1. Introduction

The enteric nervous system, comprising the myenteric and submucosal plexi, has been likened to a simplified version of the central nervous system, on the basis of its complex network-like organization and the existence of a large number of neurotransmitters. So, the isolated guinea-pig ileum has been extensively used for assessment of the acute effects of opioids and also as a model for studying the chronic action of opioids. Opioid tolerance and physical dependence can be induced at the level of the guinea-pig ileum either by in vivo chronic treatment of animals with opioid receptor agonists (Goldstein and Schulz, 1973; Schulz and Herz, 1976) or by exposing in vitro segments of excised ileum from naive animals to opioid receptor agonists (Rezvani et al., 1982; Rezvani and Way, 1988). Tolerance to opioids is reflected as a shift to the right of the concentration–response curve for the opioid-induced inhibition of neurogenic contractions of myenteric plexus-longitudinal muscle (Johnson et al., 1978; Johnson and Fleming, 1989), whereas a state of physical dependence on opioids is reflected as a contractile response on the addition of naloxone to tolerant preparations (Collier et al.,

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00389-1

1981; Cherubini et al., 1988). Morphine-induced tolerance in the myenteric plexus-longitudinal muscle involves subsensitivity to μ-opioid receptor agonists and to several inhibitory substances such as the α_2-adrenoceptor agonist clonidine and the purinergic receptor agonist 2-chloradenosine (Gillan et al., 1979; Taylor et al., 1988). Moreover, the withdrawal contracture of ileum made dependent to morphine can be suppressed by clonidine or 2-chloradenosine because of convergent pathways of dependence on these three distinct types of agonist in the final cholinergic motor neuron of the myenteric plexus of guinea-pig ileum (Collier, 1984).

The myenteric plexus-longitudinal muscle has recently been used as a functional model in which to study the mode of action of psychotropic cannabinoid receptor agonists (Pertwee et al., 1992b, 1996). In this preparation, the cannabinoid receptor agonists acting at prejunctional cholinergic terminals reduce acetylcholine release (Coutts and Pertwee, 1997) and inhibit with high potency and remarkable stereoselectivity and structure dependence the electrically evoked contractions (Pacheco et al., 1991; Kuster et al., 1993; Pertwee et al., 1992b, 1995; Pertwee and Fernando, 1996). This inhibitory effect is readily reversed by SR141716 [*N*-(piperidino)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide], (Coutts et al., 1995; Pertwee et al., 1996), a selective cannabinoid CB_1 receptor antagonist (Rinaldi-Carmona et al., 1994), suggesting that it might be mediated by cannabinoid CB_1 receptors.

On the basis of these findings it was of interest to determine whether (1) myenteric plexus-longitudinal muscle after exposure in vitro to *R*(+)-[2,3-dihydro-5-methyl-3[(morpholinyl)methyl]pyrrolo[1, 2, 3-de]-1 , 4-benzoxazinyl]-(1-naphthalenyl) methanone mesylate (WIN 55,212-2) became tolerant to this cannabinoid and subsensitive to the inhibition elicited by morphine; (2) myenteric plexus-longitudinal muscle made tolerant to morphine in vitro showed a reduced sensitivity to the inhibitory effect of the cannabinoid receptor agonist WIN 55,212-2; (3) tolerance to WIN 55,212-2 in myenteric plexus-longitudinal muscle was associated with dependence; (4) the withdrawal contracture caused by naloxone in morphine-dependent myenteric plexus-longitudinal muscle was suppressed by WIN 55,212-2.

2. Materials and methods

2.1. Animals

Female guinea-pigs (300–350 g, Dunkin–Hartley) were housed five per cage in a room with controlled temperature (22 ± 1°C), humidity (60 ± 10%) and light (12 h per day) for at least 4 days before being used. Food and water were available ad libitum. Animal care was in accordance with the Italian State regulations governing the care and treatment of laboratory animals.

2.2. Tissue preparation

Guinea-pigs were killed by a blow on the neck. The ileum was excised and kept in a Krebs solution of the following composition (millimolar): NaCl 118, KCl 4.75, KH_2PO_4 1.19, $NaHCO_3$ 25, glucose 11, $MgSO_4$ 1.2, $CaCl_2$ 2.5 and choline chloride 20 μM. The longitudinal muscle including the myenteric plexus was carefully separated from underlying circular muscle as described by Kosterlitz et al. (1970). The strips were stretched to 1 g tension in 5-ml isolated organ baths containing Krebs solution at 37°C and bubbled with a mixture of 95% O_2–5% CO_2. The intramural nerves were stimulated with rectangular pulses (1 ms duration, 100 mA constant current intensity, which corresponds to the supramaximal voltage, 0.1 Hz) passing between a platinum electrode at the bottom and a ring of platinum fixed to the top of the organ bath. The stimuli applied to the tissue preparation were generated by a 215/S Hugo Sachs Electronik KG Stimulator (March-Hugstetten, Germany) connected to a Multiplexing Pulse Booster (Ugo Basile, Comerio, VA, Italy) and the contractions were recorded isometrically using a Ugo Basile transducer and recorder. The strips were allowed to equilibrate for 1 h before inducing tolerance to morphine or WIN 55,212-2.

2.3. Assessment of tolerance

Tolerance development to morphine or WIN 55,212-2 was induced in vitro according to the method of Rezvani et al. (1983). Briefly, after an equilibration period the strips were incubated for 5 h at 37°C in the absence and presence of morphine or WIN 55,212-2. The agonist was added to a Krebs solution in a fixed concentration representing the IC_{50} (mean value calculated from the concentration–response curves obtained in previous experiments). The tissues were washed regularly at intervals of 15 min with the perfusing solution. At the end of the incubation time, the amplitude of electrically evoked contractions in untreated and treated tissue was not significantly different. Increasing concentrations of inhibitory agonists were added to the organ bath and the decrease in amplitude of contractions evoked by electrical stimulation was measured. Tolerance was evaluated by comparison of the concentration–response curves for agonist inhibition of electrically evoked contractions between segments incubated with or without agonist. Only one concentration–response curve was recorded per tissue.The bidirectional cross-tolerance between morphine and WIN 55,212-2 was measured by comparing the concentration–response curves for an agonist in preparations exposed (for 5 h at 37°C) or not to the other agonist. At the end of each experiment, the tolerant tissues were tested for the presence of physical dependence. The stimulator was switched off and after 15 min the tissue tolerant to morphine was challenged with naloxone (10^{-5} M), whereas tissue tolerant to WIN 55,212-2

was challenged with SR141716 (10^{-6} M). The contracture response elicited by these antagonists was expressed as percentage of the acetylcholine (10^{-5} M) maximum response.

In some experiments, we also tested the ability of WIN 55,212-2 (5×10^{-8} M) to reduce the withdrawal contracture in morphine-dependent tissue by adding the cannabinoid to the medium 15 min before challenge with naloxone.

2.4. Chemicals

The following compounds were used: morphine hydrochloride (Guieu Labs., Milan, Italy), naloxone hydrochloride (Sigma-Aldrich, Milan, Italy), acetylcholine chloride (Sigma-Aldrich) dissolved in distilled water; WIN 55,212-2 (Research Biochemicals International, Natick, MA) and SR141716 (Sanofi Reserche, Montpellier, France). Stock solutions of WIN 55,212-2 (2×10^{-3} M) and SR141716 (10^{-2} M) prepared in dimethylsulfoxide (DMSO) were diluted with distilled water containing one drop of Tween 80.

2.5. Analysis of data

The data reported represent mean values ± S.E.M. Inhibition of electrically induced twitch height is expressed as a percentage. Percent inhibition was calculated using the average contraction height at the maximum inhibition following the addition of the agonist, divided by the average contraction height 1 min before exposure to the initial dose of agonist. Linear regression was applied to the linear portion of the concentration–response curve and was calculated according to Steel and Torrie (1960). Differences between the slopes of tolerant and non-tolerant preparations were calculated by applying a test of parallelism to the linear regressions and the degree of tolerance was determined by calculating the rightward shift of the concentration–response curve.

Statistical comparisons of the means were performed with a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test in the case of multiple means. In all statistical comparisons, a P value less than .05 was considered significant.

3. Results

3.1. Effect of morphine on myenteric plexus-longitudinal muscle exposed to morphine

As illustrated in Fig. 1, in the myenteric plexus-longitudinal muscle exposed for 5 h to morphine, (10^{-7} M) the inhibitory effect of opiate was less than in tissue not incubated with morphine, indicating a development of tolerance. When compared with the control tissue, the concentration-curve for the inhibitory effects of morphine was shifted to the right, the slope of the linear portion of the curve was not significantly changed and the maximum response was strikingly reduced. The decrease in responsiveness with a high concentration of morphine was such that 50% inhibition could not be determined. Thus, the degree of tolerance to morphine was estimated from the magnitude of the shift calculated on the linear portion of the concentration–response curves.The loss in sensitivity, so evaluated, was about 11-fold.

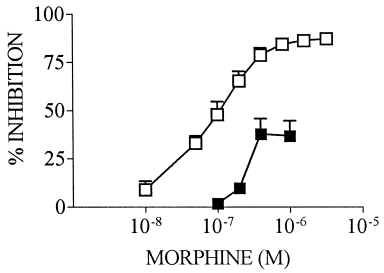

Fig. 1. Concentration–response curves for the inhibitory effect of morphine in myenteric plexus-longitudinal muscle preparations incubated (■) or not (□) for 5 h with morphine (10^{-7} M). Each point represents the mean ± S.E.M. from four or five experiments.

3.2. Effect of WIN 55,212-2 on myenteric plexus-longitudinal muscle exposed to WIN 55,212-2

Direct incubation of myenteric plexus-longitudinal muscle with WIN 55,212-2 for 5 h resulted in the development of tolerance. Segments incubated with cannabinoid agonist showed a lower sensitivity to the inhibitory effect of WIN 55,212-2 than segments incubated without drug (Fig. 2). A marked decrease in the maximum inhibitory effect of WIN 55,212-2 was found: the high concentration of cannabinoid depressed the neurogenic response only by 23%. The flattening of the concentration–response curve was such that the degree of tolerance could not be accurately determined.

3.3. Cross-tolerance between WIN 55,212-2 and morphine

The existence of cross-tolerance between WIN 55,212-2 and morphine was assessed by recording concentration–response curves for morphine in myenteric plexus-longitudinal muscle strips not exposed or exposed to WIN 55,212-2 (5×10^{-8} M) for 5 h. As shown in Fig. 3, the morphine concentration–response curve was shifted to the right in preparations incubated with WIN 55,212-2, which indicated cross-tolerance to morphine. The exposure of myenteric plexus-longitudinal muscle to the cannabinoid agonist decreased the maximum effect of morphine: in the control strip it was 78.78 ± 5.22% whereas in the WIN 55,212-2 treated-preparation it was 38.89 ± 5.11. The magnitude of tolerance to the opiate could not be measured because there was a significant difference between the slopes of

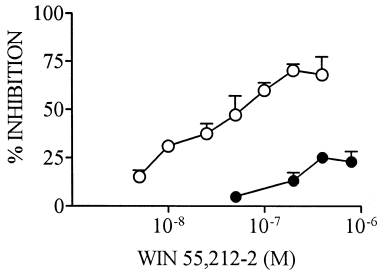

Fig. 2. Concentration–response curves for the inhibitory effect of WIN 55,212-2 in myenteric plexus-longitudinal muscle preparations incubated (●) or not (○) for 5 h with WIN 55,212-2 (5×10^{-8} M). Each point represents the mean ± S.E.M. from four or five experiments.

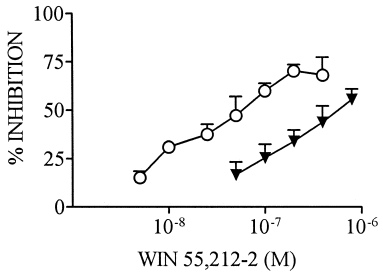

Fig. 4. Concentration–response curves for the inhibitory effect of WIN 55,212-2 in myenteric plexus-longitudinal muscle preparations incubated (▼) or not (○) for 5 h with morphine (10^{-7} M). Each point represents the mean ± S.E.M. from four or five experiments.

curves found in control and WIN 55,212-2-treated preparations.

3.4. Cross-tolerance between morphine and WIN 55,212-2

To determine the possible existence of cross-tolerance between morphine and WIN 55,212-2, the sensitivity of myenteric plexus-longitudinal muscle preparations to WIN 55,212-2 after 5 h of exposure to morphine (10^{-7} M) was evaluated in comparison to that observed in control preparations. As shown in Fig. 4 in the myenteric plexus-longitudinal muscle strips incubated with morphine, the ability of WIN 55,212-2 to inhibit the twitch response was attenuated and the cannabinoid concentration–response curve was shifted in a parallel manner to the right. In morphine-tolerant preparations, the highest WIN 55,212-2 concentration (8×10^{-7} M) depressed the neurogenic response by 55.55 ± 5.31%. This value was significantly different from the maximum effect obtained with the cannabinoid in control preparations ($P<0.05$). Cannabinoid concentrations above 8×10^{-7} M were not used as these would have produced DMSO concentrations that could themselves inhibit the twitch response of myenteric plexus-longitudinal muscle. Thus, we could not ascertain whether the tolerance to WIN 55,212-2 was accompanied by a significant reduction in maximal effect. The degree of tolerance to the cannabinoid evaluated from the shift of the linear portion of the concentration–response curve was 12-fold.

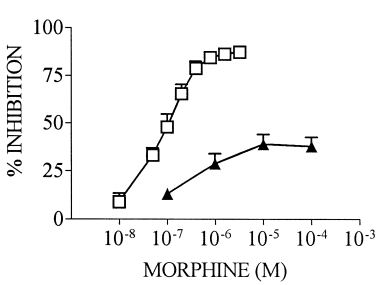

Fig. 3. Concentration–response curves for the inhibitory effect of morphine in myenteric plexus-longitudinal muscle preparations incubated (▲) or not (□) for 5 h with WIN 55,212-2 (5×10^{-8} M). Each point represents the mean ± S.E.M. from four or five experiments.

3.5. Assessment of dependence

The tissues in which tolerance to morphine or WIN 55,212-2 was determined were tested for the presence of physical dependence. The addition of naloxone (10^{-5} M) to the myenteric plexus-longitudinal muscle rendered tolerant to morphine produced a typical withdrawal contracture, whereas it was ineffective on those not previously exposed to narcotic (Fig. 5). In contrast, SR141716 at 10^{-6} M, a concentration which antagonized the inhibitory effect of WIN 55,212-2 10^{-7} M in control preparations, did not produce significant contractures in either myenteric plexus-longitudinal muscle rendered tolerant to WIN 55,212-2 or myenteric plexus-longitudinal muscle incubated without cannabinoid (data not shown). To verify the convergent dependence, myenteric plexus-longitudinal muscle tolerant to morphine 15 min before challenge with naloxone 10^{-5} M was exposed to WIN 55,212-2 5×10^{-8}

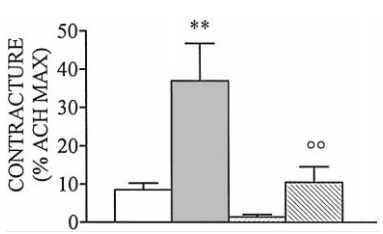

Fig. 5. Effect of WIN 55,212-2 (5×10^{-8} M) on contractures of myenteric plexus-longitudinal muscle precipitated by naloxone (10^{-5} M) following 5-h contact with morphine (10^{-7} M). The cannabinoid was added 15 min before naloxone. Naive myenteric plexus-longitudinal muscle + naloxone (rectangle); morphine myenteric plexus-longitudinal muscle + naloxone (▨); naive myenteric plexus-longitudinal muscle + WIN 55,212-2 + naloxone (rectangle with diagonal lines going from lower left to upper right); morphine myenteric plexus-longitudinal muscle + WIN 55,212-2 + naloxone (rectangle with diagonal lines going from upper left to lower right). $^{**}P<0.01$ vs. naive myenteric plexus-longitudinal muscle + naloxone; $^{\circ\circ}P<0.01$ vs. morphine myenteric plexus-longitudinal muscle + naloxone. Each bar represents the mean ± S.E.M. from 12 experiments.

M. In the absence of WIN 55,212-2 the contracture evoked by naloxone was 36.98 ± 9.83% of the maximum contracture elicited by acetylcholine 10^{-5} M, whereas in the presence of WIN 55,212-2 it was only 10.56 ± 4.03%.

4. Discussion

The results clearly indicate that when incubated for 5 h with myenteric plexus-longitudinal muscle at 37°C, WIN 55,212-2 can rapidly induce a high degree of tolerance. In preparations exposed to WIN 55,212-2, the concentration–response curve for WIN 55,212-2 was shifted to the right and the maximum inhibition evoked by the cannabinoid agonist was strikingly decreased. Earlier studies have demonstrated that tolerance to cannabinoids can be induced both in myenteric plexus-longitudinal muscle or vas deferens of MF1 mouse by in vivo administration of Δ^9-tetrahydrocannabinol (Pertwee et al., 1992a, 1994). We showed here that it is possible to induce tolerance to cannabinoids also in vitro, using the same experimental conditions which have been found valid for producing tolerance to opiates (Rezvani et al., 1983), and we propose that this procedure could be a simple alternative method for assessment of tolerance to other cannabinoids and for studying the mechanisms underlying this phenomenon. At the present time, the adaptive changes responsible for the reduced sensitivity of myenteric plexus-longitudinal muscle to the inhibitory effect of WIN 55,212-2 are unknown. However, as suggested by Pertwee (1991), the demonstration that an isolated tissue can be made tolerant to WIN 55,212-2 strengthens the hypothesis that cannabinoid tolerance is primarily pharmacodynamic in nature. It remains to be determined whether the changes in the concentration–response curves for WIN 55,212-2, such as changes in horizontal position and maximum effect, may involve alterations in receptors or transduction processes rather than in post-transductional events, sensitivity of smooth muscle to junctional neurotransmitters and responsiveness of myenteric neurons to cannabinoid receptor agonist.

Concerning the question addressed to verify the presence of the cross-tolerance between WIN 55,212-2 and morphine, we found that myenteric plexus-longitudinal muscle rendered tolerant to WIN 55,212-2 became subsensitive to the inhibitory effect of morphine. The concentration–response curve for morphine in WIN 55,212-2-treated preparations was shifted to the right compared to that obtained in naive preparations and the slope of the curve and the maximum effect were significantly decreased. In this regard, myenteric plexus-longitudinal muscle differs from mouse vas deferens since vasa deferentia taken from Δ^9-tetrahydrocannabinol-pretreated mice did not desensitize to the non-cannabinoid twitch inhibitors, morphine, [D-Ala^2, *N*-Me-Phe^4, Gly-ol^5]enkephalin, [D-Ala^2, D-Leu^5]enkephalin, U69,593 [(+)-(5α,7α,8β)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzeneacetamide] or clonidine (Pertwee and Griffin, 1995). The reasons for these differences are unknown but we suggest that they depend on the different methods used to induce the development of tolerance or, as already demonstrated for opioids (Dunman et al., 1988), to a tissue-specific adaptation. The mechanisms underlying the cross-tolerance to morphine have not yet been investigated. However, one possible explanation which could account for the change in the slope of the concentration–response curve and in morphine efficacy is a non-competitive interaction of WIN 55,212-2 with μ-opioid receptors. In fact, it has been reported that Δ^9-tetrahydrocannabinol and related cannabinoids, at pharmacologically relevant concentrations, modulate μ- and δ-opioid receptors in a non-competitive manner by acting at an allosteric site on the receptor protein (Vaysse et al., 1987). Moreover, since it is well known that WIN 55,212-2 and morphine share the same transduction mechanisms (G_i) and use a common intracellular effector, adenylate cyclase (Collier and Roy, 1974; Sharma et al., 1975; Rodbell, 1980; Pertwee, 1997), we do not exclude the possibility that treatment with WIN 55,212-2 reduces the effectiveness of the transduction processes. The cross-tolerance between WIN 55,212-2 and morphine was bidirectional. In fact, the myenteric plexus-longitudinal muscle rendered tolerant to morphine showed a decreased responsiveness to WIN 55,212-2. Incubation of this tissue for 5 h with morphine 10^{-7} M resulted in the development of 12-fold cross tolerance to WIN 55,212-2. These data agree with the results of previous experiments performed both with circular and with longitudinal muscle-myenteric plexus preparations from morphine-pretreated guinea-pigs, which exhibited tolerance to the inhibitory agonists acting via different receptors, such as μ- and κ-receptors (Garaulet et al., 1994), α-adrenoceptors and adenosine receptors (Gillan et al., 1979; Taylor et al., 1988). It has been reported that the tolerance of myenteric plexus to these agonists induced by the implantation of morphine pellets is not due to changes in the receptors or transduction processes but depends on partial depolarization of S neurons (cholinergic motor neurons innervating the longitudinal muscle) when the three types of receptors are colocalized (Meng et al., 1997). Briefly, in myenteric plexus-longitudinal muscle from naive guinea-pigs opiates, clonidine and 2-chloradenosine inhibit the electrically induced twitches of the muscle by hyperpolarization of the myenteric S neurons. In myenteric plexus-longitudinal muscle from animals chronically exposed to morphine S neurons are partially depolarized and show a non-specific subsensitivity to morphine, clonidine and 2-chloradenosine. Because of the partially depolarized state of tolerant neurons, the hyperpolarizing agent, e.g., morphine, would need to produce a greater hyperpolarization to achieve a twitch inhibition equal to that seen in naive preparations. Thus, higher concentrations of drug are required to obtain an equivalent inhibition in tolerant tissue and in control tissue. It has been reported that WIN 55,212-2 causes in some myen-

teric S neurons a reversible and stereoselective depression of fast and slow excitatory synaptic potentials (e.p.s.ps) (Lopez-Redondo et al., 1997). Thus, a higher concentration of WIN 55,212-2 may be required to obtain in morphine-tolerant tissue an equivalent depression of fast and slow e.p.s.ps. as in naive neurons.

Concerning the question about whether in myenteric plexus-longitudinal muscle the development of tolerance to WIN 55,212-2 is accompanied by the development of physical dependence, we found that SR141716 failed to produce a withdrawal contracture in tissue exposed for 5 h to WIN 55,212-2 5×10^{-8} M. There are some possible explanations for the lack of a withdrawal response to this drug. If the assumption is made that the withdrawal contracture is an indicator of dependence, then it is possible that WIN 55,212-2, at the concentration used, might not have the potential to produce dependence. Alternatively, a longer time than that necessary to induce tolerance could be required for the development of physical dependence. Moreover, SR141716 might not be the appropriate antagonist drug to precipitate the withdrawal response to WIN 55,212-2. It has been proposed, at least for opiates, that the rate of removal of opiates from their receptors is an important determinant of the magnitude of the withdrawal response (Chahl, 1986). We observed that the inhibition of electrically evoked contractions elicited by WIN 55,512-2 10^{-7} M in control preparations was completely reversed by SR141716 10^{-6} M only after 20 min. Thus, the slow displacement of WIN 55,212-2 by SR141716 could account for the lack of withdrawal response. A novel CB_1 antagonist, more potent than SR141716, could be useful in clarifying this point.

Finally, we showed here that WIN 55,212-2, as previously reported for Δ^9-tetrahydrocannabinol (Morrone et al., 1993), is able to suppress the withdrawal contracture induced by naloxone in morphine-dependent myenteric plexus-longitudinal muscle. These findings indicate that WIN 55,212-2 can substitute for morphine in opiate-dependent guinea-pig ileum preparations and suggest that the interaction between opioid and cannabinoid drugs is not restricted to sites within the central nervous system (Bhargava, 1976; Bloom and Dewey, 1978; Welch, 1993; Smith et al., 1994) but also extends to the peripheral nervous system. The inhibition of morphine withdrawal signs observed in myenteric plexus-longitudinal muscle suggests that WIN 55,212-2 may modulate the activity of neuronal pathways involved in the expression of dependence. It is well known that the abrupt contracture induced by naloxone in morphine-dependent myenteric plexus-longitudinal muscle is produced by an increased release of acetylcholine, 5-hydroxytryptamine and substance P in neuromuscular synapses (Schulz and Herz, 1976; Gintzler, 1979, 1980). However, it is now quite clear that cannabinoid receptor agonists in myenteric plexus-longitudinal muscle act presynaptically by reducing acetylcholine release (Coutts and Pertwee, 1997). Taken together, these findings suggest that one possible explanation for the inhibition of morphine dependence expression could be interference by WIN 55,212-2 of acetylcholine release. However, since it has been shown that Δ^9-tetrahydrocannabinol inhibits the binding of μ-receptor agonists (Vaysse et al., 1987), we cannot exclude the possibility that the suppression of withdrawal contraction also depends on the interaction of WIN 55,212-2 with μ-opioid receptors.

Acknowledgements

This work was supported by Murst 40% (1997) and 60% (1996) (Ministero dell'Universita' e della Ricerca Scientifica e Tecnologica).

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