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European Journal of Pharmacology 318 (1996) 221–229

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Antidepressant-like effects of CCK_B receptor antagonists: involvement of the opioid system

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Received 7 May 1996; revised 24 July 1996; accepted 27 September 1996

Abstract

RB 101 (*N*-[(*R,S*)-2-benzyl-3-[(*S*)-2-amino-4-methylthiobutyldithio]-1-oxopropyl]-L-phenylalaninebenzyl ester), a systemically active inhibitor of enkephalin catabolism, has been shown to elicit antidepressant-like effects in mice, both in the forced-swimming and in the conditioned suppression of the mobility tests. The same type of response has been also observed following administration of the cholecystokinin CCK_B receptor antagonist L-365,260 ((3*R*)-(+)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl)-3-methylphenylurea). Interestingly, the δ-opioid receptor antagonist naltrindole (17-cyclopropylmethyl-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-6,7,2′-3′-indolomorphinan) blocks the effect of both RB 101 and L-365,260 in the conditioned suppression of the motility test. In this work we have investigated the involvement of the opioid system in the antidepressant response to the CCK_B receptor antagonist L-365,260 in the forced-swimming test in mice. The effect of L-365,260 was decreased by the δ-opioid receptor antagonist naltrindole. Furthermore, the CCK_B receptor agonist, BC 264 (Boc-Tyr(OSO_3H)-gNle-mGly-Trp-(NMe)Nle-Asp-Phe-NH_2), blocked the antidepressant-like effect of RB 101 while CCK-8 (H-Asp-Tyr(OSO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2) enhanced the effect of this drug, probably through stimulation of central CCK_A receptors, since the CCK_A receptor antagonist devazepide ((3*S*)-(−)-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl)-1*H*-indole-2-carboxamide) abolished the CCK-8-induced potentiation of the RB 101 effect. In addition, RB 101 enhanced the effect of L-365,260. Such an effect was blocked by the δ-opioid receptor antagonist naltrindole. These data further support the involvement of opioid receptors in the antidepressant-type effect induced by CCK_B receptor blockers and support the hypothesis of a regulatory role of CCK in the activity of the endogenous opioid system. As in other experimental paradigms, CCK_A and CCK_B receptor stimulation appears to have opposite effects in modulating opioidergic activity.

Keywords: Cholecystokinin; CCK-8; BC 264; CCK_B receptor antagonist; L-365,260; Devazepide; CCK_A receptor antagonist; Depression; Stress; Forced-swimming test; RB 101; Enkephalin catabolism inhibitor

1. Introduction

Cholecystokinin (CCK) is a gastrointestinal peptide also found in the mammalian central nervous system (CNS). The main physiological central actions of CCK include modulation of pain perception (Baber et al., 1989) and modulation of dopaminergic activity (Crawley, 1991). CCK-8 binds to at least two different receptors: the CCK_A receptor, also called the peripheral receptor, and the CCK_B receptor, which is the most abundant form in the brain (Innis and Snyder, 1980; Moran et al., 1986).

There is evidence that some biological actions of CCK are opposite to those triggered by opioids, suggesting that CCK might function in the CNS as a physiological opioid antagonist (Faris et al., 1983; Noble et al., 1993). This is supported by the close anatomical distribution of CCK and opioid peptides through the CNS (Stengaard-Pedersen and Larson, 1981; Gall et al., 1987; Pohl et al., 1990). Moreover, CCK-8 antagonizes analgesia induced by morphine as well as by endogenous opioid peptides (Baber et al., 1989 and refs. cited therein). Accordingly CCK_B receptor

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PII S0014-2999(96)00773-X

antagonists enhance morphine as well as endogenous opioid-induced analgesia (Watkins et al., 1985a,b; Dourish et al., 1990; Maldonado et al., 1993; Valverde et al., 1994), and prevent tolerance development to morphine (Watkins et al., 1984; Dourish et al., 1990; Xu et al., 1992). It has been shown in in vitro studies that δ-opioid receptor agonists enhance and μ-opioid receptor agonists reduce the release of CCK from slices of rat substantia nigra and spinal cord (Benoliel et al., 1991, 1992). Likewise in vivo studies have shown that δ-opioid agonists stimulate the release of CCK-related peptides in whole brain (Ruiz-Gayo et al., 1992), while μ-opioid receptor agonists inhibit CCK release from the rat dorsal horn (Rodríguez and Sacristán, 1989). All these findings suggest that the antagonism between CCK and opioids may be of physiological relevance as a homeostatic mechanism in maintaining nociceptive thresholds (Faris et al., 1983).

Two different groups of findings point to an opioid involvement in depression. First, inhibitors of enkephalin-degrading enzymes have been found to be active in different models of depression (De Felipe et al., 1989; Baamonde et al., 1992; Smadja et al., 1995). Second, the effects of both classical and atypical antidepressants are partially blocked by opioid receptor antagonists (De Felipe et al., 1989; Baamonde et al., 1992). As a further indication of the physiological antagonism of opioids and CCK, the CCK_B receptor antagonist L-365,260 has been found to be active in both the forced-swimming (Hernando et al., 1994) and the conditioned suppression of the mobility tests (Derrien et al., 1994). Interestingly, the δ-opioid receptor antagonist naltrindole was shown to prevent the L-365,260 effect in the conditioned suppression of the mobility test (Derrien et al., 1994).

The aim of this work was to further investigate the involvement of the opioid system in the antidepressant-type effect elicited by the CCK_B receptor antagonist, L-365,260, in the forced-swimming test in mice. For this purpose, we have studied the effect of both δ- and μ-opioid receptor antagonists on the response to L-365,260. This was completed by a study of the effect of selective CCK receptor agonists and antagonists on the response induced by RB 101, a systemically active dual inhibitor of both neutral endopeptidase 24.11 and aminopeptidase N (Fournié-Zaluski et al., 1992; Roques et al., 1993).

2. Materials and methods

2.1. Animals

Male Swiss albino mice (Interfauna Ibérica, Spain) weighing 25–27 g were housed in groups with 12 h light/dark cycles for a week before the experiment. Food and water were available ad libitum. All experiments were carried out between 11 h and 15 h. Each animal was used only once.

2.2. Chemicals

L-365,260 ((3*R*)-(+)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl)-3-methyl phenylurea; Lotti and Chang, 1989) and devazepide ((3*S*)-(−)-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl)-1*H*-indole-2-carboxamide; Chang and Lotti, 1986) were kindly given by Merck Sharp and Dohme Research Laboratories (West Point, PA, USA). CCK-8 (H-Asp-Tyr(OSO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2) was from Bachem (Switzerland). Cremophore EL, naloxone and naltrindole (17-cyclopropylmethyl-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-6,7,2′-3′-indolomorphinan; Portoghese et al., 1988) were purchased from Sigma (St. Louis, MO, USA). β-Funaltrexamine ((*E*)-4-[[(5α,6β)-17-cyclopropylmethyl-4,5-epoxy-3,14-dihydromorphinan-6-yl]-amino]-4-oxo-2-butenoic acid methyl ester; Portoghese et al., 1980) was from RBI (Natick, MA, USA). RB 101 (*N*-[(*R*,*S*)-2-benzyl-3-[(*S*)-2-amino-4-methylthiobutyldithio]-1-oxopropyl]-L-phenylalaninebenzyl ester; Fournié-Zaluski et al., 1992) and BC 264 (Boc-Tyr(OSO_3H)-gNle-mGly-Trp-(NMe)Nle-Asp-Phe-NH_2) were synthesized as described (Charpentier et al., 1988).

2.3. Forced-swimming test procedure

The forced-swimming test was performed as described by Porsolt et al. (1977). Briefly, each mouse was placed during 6 min in a vertical Plexiglas cylinder (20 × 9.5 cm) with a water depth of 11 cm at 21–23°C. The duration of immobility, after a delay of 2 min, was measured during the last 4 min. Only swimming, but not floating movements, were taken into account for immobility measurement. Animals were not pre-tested.

2.4. Injection procedure

L-365,260 and devazepide were given i.p. 30 min before the test in 4% arabic gum containing 5% ethanol. i.c.v. devazepide was administered by free hand (Haley and McCormick, 1957) in 5 μl of a mixture of ethanol/poly (ethylene glycol) 200/saline (1:1:98) 30 min before the test. CCK-8 and BC 264 were administered i.c.v. in 5 μl saline 35 min before the test. Naltrindole and naloxone were administered s.c. in saline 35 min before the test. β-Funaltrexamine was administered i.c.v. in saline, 24 h before the test. RB 101 was given i.v. in a mixture of Cremophore EL/ethanol/water (1:1:8) 10 min before the test. Time administration for all the compounds used in this study was choosen according to previous studies (Durieux et al., 1991; Hernando et al., 1994; Derrien et al., 1994; Baamonde et al., 1992).

2.5. Analysis of data

Comparisons between groups were made by using a one-way analysis of variance (ANOVA). Post-hoc comparisons were made by using the Newman-Keuls test.

ED_{50} values and their 95% confidence limits were calculated by log-probit analysis according to the method of Litchfield and Wilcoxon (1949). For ED_{50} determination, maximal duration of immobility was set at 204 s, the average time of immobility in untreated animals. The ED_{50} was defined as the dose of the compound required to decrease the duration of the immobility to 102 s.

3. Results

3.1. Effect of opioid receptor antagonists on the response elicited by L-365,260

The effect of several opioid receptor antagonists on the antidepressant-type response elicited by L-365,260 was evaluated. As shown in Fig. 1, the δ-opioid receptor antagonist naltrindole (0.3 mg/kg s.c., 35 min before the test), prevented the effect of the CCK_B receptor antagonist L-365,260 (1 mg/kg i.p., 30 min before the test) (ANOVA $F(3,28) = 5.68$, $P < 0.05$; Newman-Keuls, $^{**}P < 0.01$ for L-365,260 as compared to the control group; $P < 0.05$ for L-365,260 as compared to the other two groups).

As illustrated in Fig. 2, the μ-opioid receptor antagonist β-funaltrexamine (i.c.v., 24 h before the test) was administered at the doses of 0.2, 2 and 20 nmol/mouse. This antagonist did not modify the effect of L-365,260 at the doses of 0.2 (ANOVA $F(3,27) = 5.02$, $P > 0.01$; Newman-Keuls, $^{*}P < 0.05$) and 2 nmol/mouse (ANOVA $F(3,35) = 2.66$, $P < 0.05$; Newman-Keuls, $^{*}P < 0.05$). However, when given at 20 nmol/mouse, β-funaltrexamine fully blocked the effect of L-365,260 (ANOVA $F(3,23) = 4.49$, $P < 0.01$; Newman-Keuls, $^{**}P < 0.01$ for the L-365,260 group compared to the other three groups). The effect of naloxone on the antidepressant-type response to L-365,260 was also tested. When given at the doses of 1 and 0.1 mg/kg, naloxone fully blocked the effect of L-365,260 (Fig. 3) (1 mg/kg ANOVA $F(3,28) = 4.78$, $P < 0.01$, Newman-Keuls, $^{*}P < 0.05$; 0.1 mg/kg ANOVA $F(3,27) = 4.80$, $P < 0.01$, Newman-Keuls, $^{*}P < 0.05$). No effect of naloxone was observed at the dose of 0.01 mg/kg.

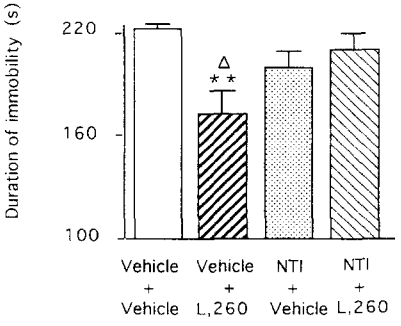

Fig. 1. Effect induced by the δ-opioid receptor antagonist naltrindole (NTI; 0.3 mg/kg s.c., 35 min before the test) on the decrease of the duration of immobility induced by L-365,260 (L,260; 1 mg/kg, i.p., 30 min before the test) in the forced-swimming test. $^{**}P < 0.01$ as compared to the vehicle group; $^{\Delta}P < 0.05$ as compared to the other two groups (Newman-Keuls test). The results are expressed as means ± S.E.M. ($n = 7$–8 animals for each group).

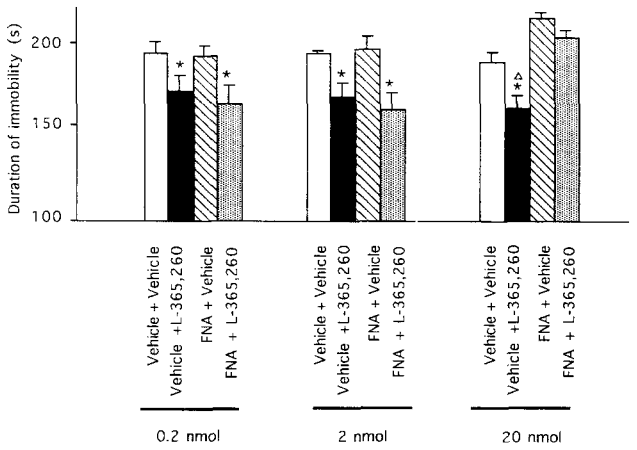

Fig. 2. Effect of the μ-opioid receptor antagonist β-funaltrexamine (FNA) on the response to L-365,260 in the forced-swimming test. FNA was given i.c.v., 24 h before the test, in a dose of 0.2, 2 or 20 nmol/mouse to animals receiving 1 mg/kg of the CCK_B receptor antagonist L-365,260 (i.p., 30 min before the test). The results are expressed as means ± S.E.M. ($n = 7$–8 animals for each group). $^{*}P < 0.05$ as compared to the vehicle (open columns) and to the FNA group (hatched columns). $^{\Delta}P < 0.05$, compared to the other three groups (Newman-Keuls test).

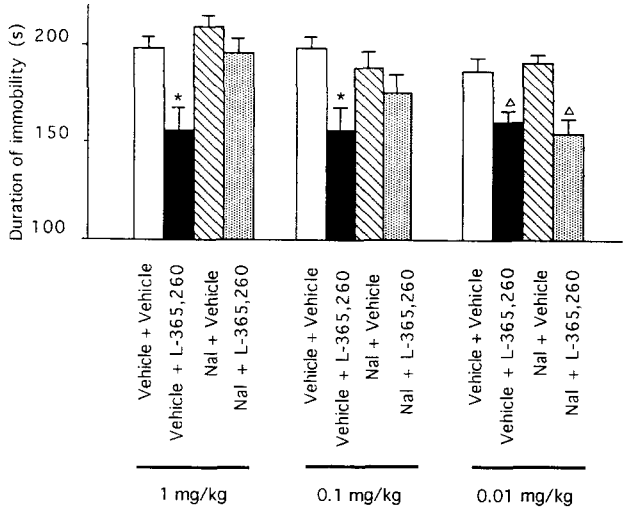

Fig. 3. Effect of the opioid receptor antagonist naloxone (Nal; s.c., 35 min before the test) on the response elicited by L-365,260 (i.p., 30 min before the test) in the forced-swimming test. Naloxone was given in a dose of 1, 0.1 or 0.01 mg/kg to mice receiving 1 mg/kg of the CCK_B receptor antagonist L-365,260. The results are expressed as means ± S.E.M. ($n = 7$–10 animals for each group). $^{*}P < 0.05$ compared to the other three groups; $^{\Delta}P < 0.05$ compared to the other two groups (Newman-Keuls test).

3.2. Effect of CCK receptor agonists on the response induced by the mixed inhibitor of enkephalin catabolism RB 101

The effect of CCK-8 as well as BC 264, a selective CCK_B receptor agonist, on the response to RB 101 was studied. As illustrated in Fig. 4a, administration of 10 pmol (i.c.v., 30 min before the test) of BC 264 prevented the effect of RB 101 (5 mg/kg i.v., 10 min before the test) (ANOVA $F(3,29) = 6.26$, $P < 0.05$; Newman-Keuls, $^{**}P < 0.01$ for RB 101 as compared to vehicle controls and BC 264 groups; $P < 0.05$ for RB 101 as compared to BC 264 + RB 101 group). In contrast to this result, the nonselective CCK receptor agonist CCK-8, i.c.v. administered (1 nmol/mouse, 30 min before the test) enhanced the effect of the same dose of RB 101 (ANOVA $F(3,37) = 23.65$, $P < 0.01$; Newman-Keuls $^{**}P < 0.01$ for both RB 101 and RB 101 + CCK-8 as compared to vehicle controls and CCK-8 groups; $P < 0.05$ for RB 101 + CCK-8 as compared to RB 101 group) (Fig. 4b).

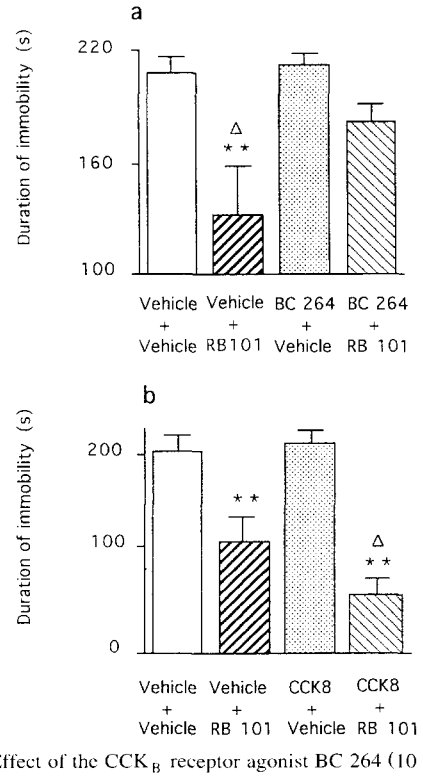

Fig. 4. (a) Effect of the CCK_B receptor agonist BC 264 (10 pmol/mouse i.c.v., 30 min before the test) on the response to the inhibitor of enkephalin catabolism RB 101 (5 mg/kg i.v., 10 min before the test) in the forced-swimming test. The results are expressed as means ± S.E.M. ($n = 8$ mice for each group). $^{**}P < 0.01$ as compared to the vehicle and the BC 264 groups, $^{\Delta}P < 0.05$ as compared to the BC 264 + RB 101 group (Newman-Keuls test). (b) Effect of CCK-8 (1 nmol/mouse i.c.v., 30 min before the test) on the antidepressant-like response elicited by RB 101 (5 mg/kg i.v., 10 min before the test) in the forced-swimming test. The results are expressed as means ± S.E.M. ($n = 8$–10 animals). $^{**}P < 0.01$ as compared to control and CCK-8 groups, $^{\Delta}P < 0.05$ as compared to the RB 101 group (Newman-Keuls test).

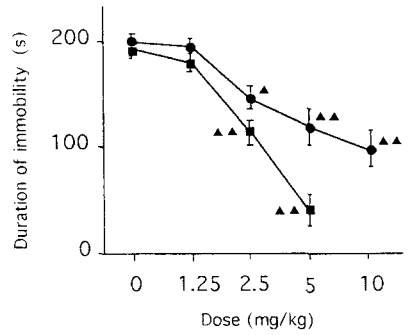

Fig. 5. Dose-response plot for both RB 101 (■) and the association RB 101 + CCK-8 (●) in the forced-swimming test. RB 101 was administered i.v. at the doses indicated, 10 min before the test. CCK-8 was administered i.c.v. at the dose of 1 nmol/mouse. Each value represents the mean ± S.E.M. of 8–12 animals. $^{\blacktriangle}P < 0.05$, $^{\blacktriangle\blacktriangle}P < 0.01$, Newman-Keuls test.

3.3. Enhancement of RB 101 antidepressant-type responses by CCK-8

The ability of CCK-8 (i.c.v., 1 nmol/mouse 30 min before the test) to displace the dose-response curve observed 10 min after i.v. RB 101 administration (1.25, 2.5, 5 and 10 mg/kg) was investigated. One-way ANOVA revealed a significant effect for RB 101 ($F(4,60) = 3.83$; $P < 0.01$) and for RB 101 + CCK-8 ($F(3,38) = 4.51$; $P < 0.01$) treatments. CCK-8 had no effect when singly administered (Fig. 5). ED_{50} values of decrease of immobility were 8.03 mg/kg (4.68–13.76) and 2.59 mg/kg (1.49–4.51) for RB 101 and RB 101 + CCK-8 respectively (Table 1).

The ability of the CCK_A receptor antagonist devazepide (i.c.v., 1.5 nmol/mouse, 30 min before the test co-administered with CCK-8) to antagonize the potentiation of the RB 101 (2.5 mg/kg i.v.) antidepressant-type effect by CCK-8 (1 nmol/mouse i.c.v.) was studied (Table 2). One-way ANOVA showed a significant effect for the antagonism ($F(7,76) = 3.12$; $P < 0.01$; Newman-Keuls test: $^{*}P < 0.05$ for RB 101 + CCK-8 group vs. the other groups).

3.4. Enhancement of RB 101 antidepressant-type effect by L-365,260

The ability of the CCK_B receptor antagonist L-365,260 (i.p., 30 min before the test) to enhance the effect of RB

Table 1
ED_{50} values for RB 101 alone and associated with CCK-8 in the forced-swimming test

Treatment	ED_{50} (95% confidence limits) RB 101 (mg/kg)
RB 101 + saline	8.03 (4.68–13.77)
RB 101 + CCK-8	2.59 (1.48– 4.51)

Table 2
Antagonism by devazepide (1.5 nmol/mouse i.c.v.) of the potentiation of the effect of RB 101 (1.5 mg/kg i.v.) by CCK-8 (1 nmol/mouse i.c.v.)

Treatment	Duration of immobility (s)
Control	193.7 ± 8.1
CCK-8 + vehicle	174.6 ± 11.1
Devazepide + vehicle	184.3 ± 8.1
Vehicle + RB 101	182.3 ± 9.7
CCK-8 + RB 101	129.6 ± 11.7 [a]
Devazepide + RB 101	185.3 ± 10.5
Devazepide + CCK-8 + vehicle	179.2 ± 17.4
Devazepide + CCK-8 + RB 101	177.6 ± 11.7

Values are mean ± S.E.M. ($n = 8$–10 animals per group). [a] $P < 0.05$ as compared to the other groups (Newman-Keuls test).

101 (1.5 mg/kg i.v., 10 min before the test) in the forced-swimming test was tested at the unique dose of 0.5 mg/kg. One-way ANOVA revealed a significant effect for RB 101 + L-365,260 treatment (ANOVA $F(3,25) = 3.14$, $P < 0.05$; Newman-Keuls, $^{*}P < 0.05$). Such an effect was prevented by naltrindole, given at the dose of 0.3 mg/kg (s.c., 35 min before the test) (ANOVA $F(5,52) = 14.08$, $P < 0.05$; Newman-Keuls, $^{*}P < 0.05$) (Fig. 6).

3.5. Effect of devazepide on the response elicited by RB 101

As illustrated in Fig. 7, the CCK_A receptor antagonist, devazepide (1 mg/kg i.p., 30 min before the test), blocked the antidepressant-type effect elicited by RB 101 (5 mg/kg

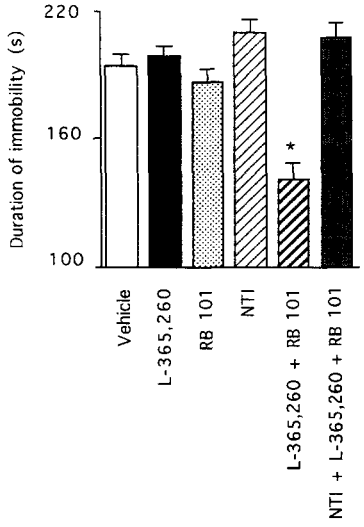

Fig. 6. Association of both RB 101 and L-365,260 results in an antidepressant-like effect in the forced-swimming test. Both RB 101 (1.5 mg/kg i.v., 10 min before the test) and L-365,260 (0.5 mg/kg i.p., 30 min before the test) were ineffective when singly administered. The δ-opioid receptor antagonist naltrindole (NTI; 0.3 mg/kg s.c., 35 min before the test) blocks the potentiation between both RB 101 and L-365,260. The results are expressed as means ± S.E.M. ($n = 8$–10 animals for each group). $^{*}P < 0.05$ as compared to the other groups (Newman-Keuls test).

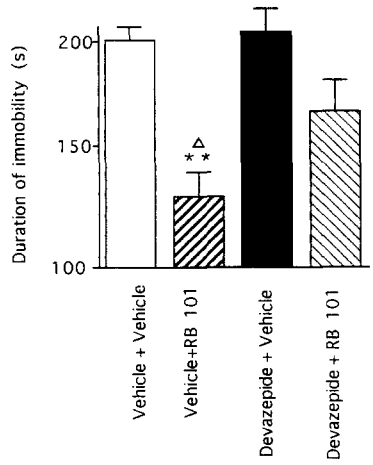

Fig. 7. Effect of the CCK_A receptor antagonist, devazepide (1 mg/kg i.p., 30 min before the test) on the response elicited by RB 101 (5 mg/kg i.v., 10 min before the test). The results are expressed as means ± S.E.M. of 6–8 animals. $^{**}P < 0.01$ compared to control and devazepide groups, $^{\Delta}P < 0.05$ compared to RB 101 + devazepide group.

i.v., 10 min before the test) (ANOVA $F(3,26) = 10.73$, $P < 0.01$; Newman-Keuls, $^{**}P < 0.01$, $^{\Delta}P < 0.05$).

4. Discussion

The interaction between CCK-ergic and opioidergic neuronal systems has been investigated in the forced-swimming test. In this test, animals react by an initial attempt to escape followed by a prolonged period of immobility. The duration of immobility is decreased by antidepressants but not by anxiolytic drugs (review in Willner, 1990). Recently, it has been reported that both RB 101, a systemically active inhibitor of enkephalin-degrading enzymes (Baamonde et al., 1992), as well as L-365,260, a selective antagonist of CCK_B receptors (Hernando et al., 1994) elicit antidepressant-type effects in this test. We show in this study that the δ-opioid receptor antagonist naltrindole prevents the antidepressant-type effect of L-365,260 in the forced-swimming test, as previously demonstrated in the conditioned suppression of the mobility test (Derrien et al., 1994). In this test mice are placed in a cage with a metallic grid floor. On the first day animals receive a series of electric footshocks. On the second day, mice are placed in the same cage without receiving electric footshocks and locomotor activity is measured. On the first day, the mice in the control group are treated in the same way as those in the conditioned suppression group except that they receive no electric footshocks. Under these conditions, conditioned animals display a marked decrease of locomotor activity, that is prevented by antidepressant drugs.

Doses of L-365,260 (1 or 0.5 mg/kg) and devazepide (1 mg/kg) were chosen according to previous studies (Dourish et al., 1989; O'Neill et al., 1991; Vasar et al., 1991; Hendrie et al., 1993; Hernando et al., 1994; Derrien

et al., 1994) that shown that both L-365,260 and devazepide elicit central effects in the range of doses used by us. This is striking inasmuch as (i) the ED_{50} for L-365,260, against [^{125}I]CCK-8, calculated in ex vivo binding experiments, is about 12 mg/kg i.v. (Patel et al., 1994), and (ii) a dose of 40 mg/kg i.p. does not inhibit, in a significant manner, the in vivo binding of [^{3}H]pBC 264 (a selective CCK_B receptor agonist) in mouse brain (Durieux et al., 1991). However, there is an important methodological difference between these studies and our present work, that could account for the differences observed. In our case L-365,260 or devazepide only competes with the endogenous ligand for CCK binding sites, and it can be speculated that low doses of antagonists are sufficient to reverse the effect of endogenous CCK.

The dose of the δ-opioid receptor antagonist naltrindole (0.3 mg/kg s.c.) that blocked the antidepressant-like effect of L-365,260 (Fig. 1) can be considered as selective for δ-opioid receptors since it did not antagonize the increase in the jump latency induced by morphine in the hot-plate test (data not shown), which is considered as a supraspinal response to nociceptive stimuli that mainly involves μ-opioid receptors (Gacel et al., 1981; Chang et al., 1982; Chaillet et al., 1984; Fang et al., 1986; Baamonde et al., 1991; Noble et al., 1992). This dose is in the range of doses used for δ-opioid receptor blocking (Portoghese et al., 1988; Jackson et al., 1989; Baamonde et al., 1992).

The μ-selective opioid receptor antagonist β-funaltrexamine was not effective in blocking the L-365,260 effect in a dose range (0.2–2 nmol/mouse) reported as active in blocking μ-mediated responses (Ward et al., 1982) but blocked the effect of L-365,260 when given at 20 nmol/mouse (Fig. 2). Naloxone given at the dose of 1 or 0.1 mg/kg, but not at 0.01 mg/kg, fully blocked the effect of L-365,260 in the forced-swimming test (Fig. 3). Even at the lowest dose, in our hands, naloxone blocked partially the effect of morphine in the hot plate test (data not shown). These data, taken together, indicate that the antidepressant-like response elicited by L-365,260 could be related with the activation of δ-opioid receptors. The involvement of opioid receptors in the response to antidepressants and to enkephalin catabolism inhibitors in the forced-swimming test has been previously reported (De Felipe et al., 1989; Baamonde et al., 1992). Moreover, antidepressant treatment led to an increase of [Met]- and [Leu]enkephalin immunoreactivity in the rat nucleus accumbens and striatum (De Felipe et al., 1985). Furthermore, opioid receptors seem to be upregulated after chronic administration of antidepressant drugs (Antkiewicz-Michaluk et al., 1984; Hamon et al., 1987).

In agreement with the results reported by Smadja et al. (1995) in the conditioned suppression of the mobility test, the effect of the enkephalin catabolism inhibitor RB 101 (Fournié-Zaluski et al., 1992) was shown to be prevented by the CCK_B receptor agonist BC 264 (Fig. 4a). The dose used (10 pmol/mouse) was chosen on the basis of its in vivo apparent affinity for mouse CCK_B receptors (Durieux et al., 1991). As previously reported (Hernando et al., 1994), this dose also antagonized the antidepressant-like effect of L-365,260 in the forced-swimming test. It must be noticed that BC 264 has been shown to elicit depressant-like effects in mice in the conditioned suppression of the mobility test at doses lacking of any action on motor activity (Derrien et al., 1994; Smadja et al., 1995). In contrast, the response to RB 101 was potentiated by i.c.v. administered CCK-8 (Fig. 4b and Table 1). This effect was fully blocked by the selective CCK_A receptor antagonist devazepide, i.c.v. administered at a dose (1.5 nmol/mouse) selective for brain CCK_A binding sites (Durieux et al., 1991). These results suggest that the activity of enkephalinergic pathways could be modulated, in an opposed manner by CCK through both CCK_A and CCK_B binding sites (Noble et al., 1993).

Thus, stimulation of CCK_A receptors might result in the activation of enkephalinergic fibers involved in the response to antidepressant drugs in the forced-swimmig test, while the activation of CCK_B receptors could lead to the inhibition of the same pathways. This hypothesis is supported by the enhancement, blocked by the δ-opioid receptor antagonist naltrindole, of the RB 101 effect (1.5 mg/kg) by a subeffective dose (0.5 mg/kg) of L-365,260 (Fig. 6) as well as by the antagonism elicited by devazepide on the antidepressant-type effect of RB 101 (Fig. 7). Our hypothesis agrees well with previous findings showing a similar regulatory mechanism between CCK and opioids in nociceptive transmission (Noble et al., 1993) and emotional control (Daugé and Roques, 1995). On the other hand, the potentiation of the effect of RB 101 by CCK-8 could be related to an increase of dopamine turnover in discrete brain areas. In this regard, it has been shown that the activation of CCK_A receptors potentiates dopamine-induced behaviours (Crawley et al., 1985) as well as dopamine-dependent adenylylcyclase activity (Studler et al., 1986) in the rat posterior nucleus accumbens. Moreover, locally injected in this latter structure CCK-8 has been shown to increase dopamine release in a microdialysis study performed in freely moving rats (Ladurelle et al., 1993). On the other hand, it is well known that in this structure dopamine release is also evoked by opioid agonists, probably through δ-opioid binding sites (Longoni et al., 1989; Daugé et al., 1992). Thus it is possible that in our experimental model the simultaneous activation of both CCK_A and δ-opioid receptors could synergically increase dopaminergic activity in limbic areas involved in dopamine-mediated antidepressant responses (Carboni et al., 1989). In fact, it has been shown that dopaminergic pathways are involved in the antidepressant-type responses triggered by both enkephalin catabolism inhibitors (Baamonde et al., 1992) and CCK_B receptor antagonists (Hernando et al., 1994).

These results indicate that the hypothesis initially proposed by Faris et al. (1983) suggesting that CCK-ergic and

opioidergic pathways might be linked in a homeostatic mechanism aimed at maintaining nociceptive thresholds, could be extended to neuronal circuits regulating adaptative behaviours to stress. Stress adaptation appears to be a critical process in the triggering of depressive states. Opioid peptides are known to be released in the CNS in response to physical (Akil et al., 1976) and psychological stressors (Nabeshima et al., 1988). Stress also provokes the release of CCK-related peptides in limbic areas (Siegel et al., 1985) as well as in discrete hypothalamic nuclei involved in the neuroendocrine response to stress (Siegel et al., 1987). It can be speculated that in a stressful situation, such as the forced-swimming test, enkephalins might facilitate the release of CCK, which in turn could inhibit enkephalin release, probably by acting upon CCK_B type receptors. The decrease in the extracellular enkephalin level might result in a depressive-like state, i.e., 'despaired behaviour' (immobility) in the forced-swimming test (Ben Natan et al., 1984). In accordance with this hypothesis, the antidepressant effect of L-365,260 may be due to the inhibition of the feedback loop regulating the activity of the enkephalinergic system.

Alternatively, CCK is now considered as a potential secretagogue of both CRH (Kamilaris et al., 1992) and ACTH (Reisine and Jensen, 1986). In consequence, it can be argued that, during stress, the blocking of CCK receptors might lead to a decrease in the activity of the hypothalamic-pituitary-adrenocortical axis, which could also be related to the antidepressant-like properties of CCK_B antagonists. In fact it has been reported that corticosterone facilitates the incorporation of information post-stress in rats, which seems to be an important event in the development of depressive syndromes (Veldhuis et al., 1985). On the other hand, a high percentage of depressive patients show high levels of plasma cortisol, which return to normal when the psychological derangement has been overcome (Hanin et al., 1985).

Acknowledgements

This work was supported by grants (SAF 924/92) from the CICYT; Ministerio de Educación y Ciencia and from the Comunidad de Madrid (241/92), Spain and by a Concerted Action (No. BMH1-CT93-1621) from the EU. F.H. is a recipient of a research Fellowship from the Comunidad de Madrid. We express our appreciation to Dr. Lotti of Merck Sharp and Dohme Research Laboratory for supplying both devazepide and L-365,260. We thank Dr. Florence Noble for critical reading of the manuscript.

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