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Antiaversive Properties of Opioids in the Conditioned Taste Aversion Test in the Rat

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BLANCQUAERT, J -P , R A LEFEBVRE AND J L WILLEMS *Antiaversive properties of opioids in the conditioned taste aversion test in the rat* PHARMACOL BIOCHEM BEHAV 27(3) 437–441, 1987 —The antiaversive effect of μ-, κ- and δ-opioid receptor agonists against conditioned taste aversion (CTA) induced by apomorphine, lithium chloride and copper sulphate in the rat was studied, in order to evaluate whether prevention of CTA is a suitable model for the study of antiemetics Anti-aversion was not a general characteristic of all opioid substances tested Only one dose of the μ-agonist morphine and only one dose of the κ-agonist ethylketocyclazocine had a consistent antiaversive effect against CTA induced by apomorphine, one dose of the δ-agonist [D-Ala², Met⁵]enkephalinamide antagonized the aversion induced by lithium chloride As the results do not correspond to our previous findings on the antiemetic effects of these opioids in the dog (all μ- and κ-agonists tested having an antiemetic effect), we conclude that the CTA test cannot be used as a screening test for potentially antiemetic drugs

CTA Apomorphine LiCl $CuSO_4$ μ-Agonists κ-Agonists δ-Agonists Aversive and antiaversive effects Antiemetic effect

IN the cat [12] as well as in the dog [28], some opioid agents have a dual effect with regard to emesis, inducing and preventing emesis We previously reported that in the dog, the antiemetic effect of opioids is mediated by μ- and/or κ-receptors and their emetic effect by δ-receptors [6]

Conditioned taste aversion (CTA) in the rat, an animal that cannot vomit [15], is considered as one of the non-emesis behavioral assays that can be used for the study of emesis and antiemetics [20] In the CTA test, an unconditioned stimulus (UCS), e g , an emetic drug, is paired with a conditioned stimulus (CS), most frequently a saccharine or sucrose solution The noxious or nauseous effect of the UCS is then associated with the CS, resulting in an aversion for the CS Some substances administered in between the presentation of the CS and the administration of the UCS prevent the aversive action of the UCS they are antiaversive An antiaversive action in the CTA test might be an indication for an antiemetic action in the emesis test

In order to evaluate this CTA test as a screening test for potentially antiemetic drugs, we tested opioid compounds with a known antiemetic and/or emetic effect in the dog [6] for their antiaversive effect in the CTA test in the rat Based on the lack of similarity between the antiemetic and antiaversive effect of the opioids, we reject the CTA test for this purpose Preliminary results of these experiments have been presented [4,5]

METHOD

Rats

Adult rats derived from a Wistar strain of the breeding centre of the University of Leuven (Belgium) and raised in our laboratory were used They were maintained at about 22°C on a 12 12 hr light/dark cycle (light from 6 a m) The weight of the rats used throughout the study varied from 100 g to 460 g, however, the weight range of the rats within each experimental group of 6 rats (females or males) was much smaller (mean±60 g) Each experimental group was used only once

Experimental Procedure

The procedure followed—a repetitive association of CS-UCS—was based on that used by Cairnie and Leach [7] The rats were housed singly and trained during 7 days to drink water during 30 min per day only They were at random assigned to an experimental group and remained in their original cage during the course of the experiment From day 7 on the rats were allowed to drink water during 30 min in the early light phase (from 9 to 9 30 or from 9 30 to 10 a.m) From day 8 on the water consumption was recorded daily by weighing the bottles (±1 g) When rats failed to drink more than 5 g/day during two consecutive days, they were dis-

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TABLE 1

INFLUENCE OF THE μ-AGONISTS ON THE AVERSIVE EFFECT OF APO, LiCl, AND $CuSO_4$

μ-Agonists (mg/kg IP)	Against APO (30 mg/kg IP)	Against LiCl (70 mg/kg IP)	Against $CuSO_4$ (100 mg/kg IG)
Morphine			
1	+16 3	+0 8	+13 3
3	+50 3*/+15 7*	−10 7	−4 6
10	+6 4	−16 0	+7 4
30	+4 7	−27 6	+15 3
Fentanyl			
0 003	+3 1	+16 1	+8 0
0 01	+7 1	−1 9	+30 7*/−23 9 (5)
0 03	+2 1	−2 3	+19 7
0 1	+17 6	+6 0	−5 4
Methadone			
1	−0 3	+18 6	+25 9
3	+27 8*/−2 6	−12 4	−27 8

For each combination of an opioid with an UCS (APO, LiCl or $CuSO_4$), the mean difference in sucrose intake (%) on the test day is given in comparison with the corresponding aversive control group The number of rats per group was six except if otherwise indicated in parentheses Two figures indicate that this dose was tested again in another series

*$p<0$ 05, significant difference in comparison with the aversive control group

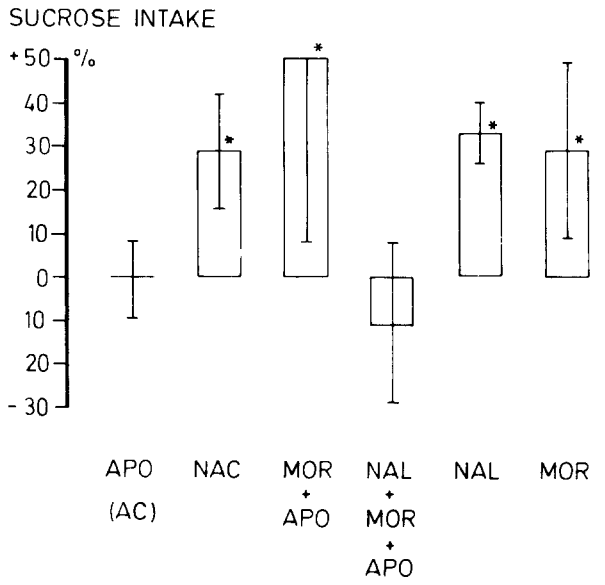

FIG 1 Antiaversive effect of morphine (3 mg/kg, MOR) against the aversion induced by apomorphine (30 mg/kg, APO) and the influence thereupon of naloxone (1 mg/kg, NAL) Differences in sucrose intake (%, mean±S D) on the test day are given in comparison with the intake of the aversive control (AC) group (*$p<0$ 05, significant difference) Naloxone and morphine, administered alone, had no effect on their own as the sucrose intake was similar to that in the non-aversive control (NAC) group receiving saline

carded On days 11, 14, 18 (conditioning days) and 21 (test day) the water was replaced by a 10% sucrose solution in water

Drug Administration

On the conditioning days the sucrose presentation was followed by drug administration The unconditioned stimuli apomorphine (APO), lithium chloride (LiCl) and copper sulphate ($CuSO_4$), or their vehicle (saline for APO and LiCl, distilled water for $CuSO_4$) were administered 30 min after the end of the sucrose presentation When opioids were given, they were injected 15 min before the UCS at each conditioning day To study the influence of naloxone on the antiaversive effect of the opioids, it was injected immediately after the sucrose presentation, 15 and 30 min before the administration of the opioid and the UCS, respectively

The experiments were performed in series of 5 to 10 experimental groups (n=6 rats in each group) A series also always contained a group only receiving the UCS and one only receiving the vehicle of the UCS

Each dose of an opioid and each combination opioid-UCS was studied in one experimental group A dose of an opioid, that was antiaversive was studied again in another experimental group All drugs were injected intraperitoneally (IP) in a volume of 0 1 to 0 3 ml/100 g except $CuSO_4$ and its vehicle, which were given intragastrically (IG) in a volume of 0 5 ml/100 g

Aversive Effect of the UCS APO, LiCl and $CuSO_4$

To determine whether or not the UCS had an aversive effect, the sucrose intake on the test day of the experimental group treated with the UCS (aversive control, AC) was compared with that of the experimental group treated with the vehicle of the UCS (non-aversive control, NAC) If the sucrose intake of the AC group was significantly ($p\leq 0$ 05) lower than the sucrose intake of the NAC group, an aversive effect was accepted Series where no aversive effect of the UCS was observed were discarded

Antiaversive Effect of the Opioids

An opioid was considered antiaversive if it prevented the aversive effect of the UCS, i e , when the sucrose intake on the test day of the rats pretreated with an opioid was significantly higher than the sucrose intake of the AC group An opioid had a potentiating effect when the sucrose intake on the test day was significantly lower than in the AC group It was accepted that an antiaversive effect of an opioid was blocked by naloxone when the sucrose intake at the test day was not significantly different from that of the AC group

Drugs Used

The following drugs were used commercially available ampoules of apomorphine HCl and morphine HCl (Sterop, Brussels, Belgium or Federa, Brussels, Belgium), fentanyl and domperidone (Janssen Pharmaceutica, Beerse, Belgium), methadone (ICI, Destelbergen, Belgium) and naloxone HCl (Dupont Pharmaceuticals, Brussels, Belgium)

[D-Ala^2, Met^5]enkephalinamide (DALA) and [Leu^5]enkephalin (UCB, Brussels, Belgium), bremazocine (courtesy of Sandoz, Basel, Switzerland), ethylketocyclazocine methanesulphonate (courtesy of Sterling-Winthrop, Rensselaer, NY), lithium chloride and copper sulphate (Merck,

TABLE 2

INFLUENCE OF THE κ-AGONISTS ON THE AVERSIVE EFFECT OF APO, LiCl AND $CuSO_4$

κ-Agonists (mg/kg IP)	Against APO (30 mg/kg IP)	Against LiCl (70 mg/kg IP)	Against $CuSO_4$ (100 mg/kg IG)
Bremazocine			
0 001	+25 2	+0 2	−8 7
0 01	+23 7	−6 2	+8 1
0 1	+16 4	−29 9*	+3 7
1	−28 4* (5)	−43 4*	−27 5
EKC			
0 001	+3 3	−13 0	−19 8
0 01	+23 1	−9 2	+5 2
0 1	+24 0*/+29 0*	+1 1	−16 5
1	+21 1	−17 4	−0 9

Explanation as for Table 1

TABLE 3

INFLUENCE OF THE δ-AGONISTS ON THE AVERSIVE EFFECT OF APO, LiCl AND $CuSO_4$

δ-Agonists (mg/kg IP)	Against APO (30 mg/kg IP)	Against LiCl (70 mg/kg IP)	Against $CuSO_4$ (100 mg/kg IG)
DALA			
0 001	+19 5	+11 2	−0 2
0 01	−1 8	+1 0	+0 6
0 1	+5 8	+22 9*/+52 8*	−17 7
1	−13 3	+16 9	−38 3*
[Leu^5]-enkephalin			
0 001	+17 2	−11 3	−0 9
0 01	−2 2	+5 6	+14 6
0 1	−0 6	+12 8	+10 7
1	+36 9	−3 6	−0 5

Explanation as for Table 1

Darmstadt, West-Germany) All these drugs were dissolved in saline with the exception of copper sulphate which was dissolved in distilled water

Analysis of Results

The sucrose intake on the test day was expressed in % of the intake on the first conditioning day, the mean±SD per experimental group (n=6 rats unless otherwise indicated) is given The two-sample rank test (Mann Whitney U-test) was used to compare the sucrose intake on the test day in the experimental groups Significance was accepted for $p \leq 0\ 05$

RESULTS

Aversive Effect of the UCS

APO (30 mg/kg) was used as UCS to test the antiaversive effect of the opioids The mean sucrose intake on the test day in 14 AC groups (treated with APO 30 mg/kg only) was 73 0% (minimum 28 5±27 4%, maximum 95 1±39 6%) Except for two series, which were rejected, the sucrose intake in the AC groups was significantly lower than in the NAC groups treated with saline (mean sucrose intake on the test day 115 2%, minimum 82 0±13 6%, maximum 132 5±32 8%) LiCl (70 mg/kg), used as UCS, decreased the sucrose intake on the test day to 45 1% (n=7 groups, minimum 15 8±12 1%, maximum 68 7±15 2%) of the initial intake In all series LiCl had an aversive effect (mean sucrose intake in the corresponding NAC groups 120 0%, minimum 91 0±8 0%, maximum 165 1±30 8%) In the AC groups of 9 series, $CuSO_4$ (100 mg/kg intragastrically) decreased the sucrose intake on the test day to 78 8% (minimum 61 7±24 8%, maximum 92 0±34 6%) of the initial intake In only 2 series this dose had no aversive effect when comparing the sucrose intake with that of the corresponding NAC groups, treated with distilled water (mean sucrose intake 118 8%, minimum 100 7±4 8%, maximum 135 6±59 4%)

Antiaversive Effect of the Opioids

μ-Agonists The μ-agonists morphine, fentanyl and methadone were tested in different doses against the UCS (Table 1) Morphine (3 mg/kg) had an antiaversive effect against the CTA induced by APO (Fig 1), this was confirmed in a second group of 6 rats The higher doses of morphine did not influence the aversive effect of APO

The antiaversive effect of morphine (3 mg/kg) was antagonized by naloxone (1 mg/kg) (Fig 1), this dose of naloxone had no effect on the aversion induced by APO and did not induce CTA by itself Morphine had no effect on the CTA induced by LiCl or by $CuSO_4$

Fentanyl (0 003 to 0 1 mg/kg) had no antiaversive effect on CTA induced by APO, increasing the dose of fentanyl to 0 3 mg/kg was lethal in 5 rats out of 6 Fentanyl had no influence on CTA induced by LiCl Fentanyl (0 01 mg/kg) had an antiaversive effect on CTA induced by $CuSO_4$, this could however not be confirmed in another group of 5 rats

Methadone, 3 mg/kg, had an antiaversive effect on the CTA induced by APO in one experimental group, this could not be confirmed in a second group With higher doses of methadone (10 and 30 mg/kg), respectively 2 and 5 rats out of 6 died during the experiment Methadone had no influence on the aversion induced by LiCl

κ-Agonists The κ-agonists bremazocine and ethylketocyclazocine (EKC) were tested in different doses against the aversion induced by the UCS (Table 2) Bremazocine had no antiaversive effect against the CTA induced by APO, LiCl or $CuSO_4$ The aversion induced by APO and LiCl however was potentiated by bremazocine (1 mg/kg, and 0 1 and 1 mg/kg respectively)

EKC (0 1 mg/kg) had an antiaversive effect against CTA induced by APO in a first experimental group a sucrose intake still significantly lower than in the NAC was observed, in a second experimental group this difference was absent This antiaversive effect was blocked by naloxone (1 mg/kg) (NAC 110 8±10 1%, AC 28 5±27 4%, naloxone + EKC + APO 44 2±17 0%) EKC had no effect on the aversion induced by LiCl and $CuSO_4$

δ-Agonists The δ-agonists [D-Ala^2, Met^5]enkephalinamide (DALA) and [Leu^5]enkephalin were tested in different doses against the aversion induced by the UCS (Table 3) DALA (0 1 mg/kg) had an antiaversive effect against the CTA by LiCl, this was confirmed in a second

experimental group In comparison with the NAC, a significantly lower sucrose intake was observed DALA had no antiaversive effect against the CTA by APO and $CuSO_4$ but at a dose of 1 mg/kg, it potentiated the aversion by $CuSO_4$ [Leu^5]enkephalin had no influence on the aversion induced by APO, LiCl or $CuSO_4$

DISCUSSION

In this study, the CTA test in the rat was evaluated as possible screening test for potentially antiemetic drugs

Aversive Stimuli

It has been shown that antiemetic opioid compounds prevent the emetic response to different emetic stimuli, which initiate the vomiting reflex at different sites, this has been explained by localizing the antiemetic action of the opioids in the coordinating vomiting centre [12] The antiaversive properties of the opioids have therefore to be studied against different UCS, initiating the CTA development via different sites We used apomorphine and $CuSO_4$, which had been used as emetics in our experiments in the dog [6] and LiCl, which is frequently used in CTA studies [1, 22, 24] and has also emetic properties [26] It is assumed that APO, LiCl and $CuSO_4$ exert their aversive effect via different pathways [11, 21–23]

The doses of APO and LiCl used correspond to those reported elsewhere [13,17] For $CuSO_4$ however, the dose used is 20 times higher than that frequently used by others [11,23] In the dose used LiCl always elicited an aversion for sucrose, APO and $CuSO_4$ failed twice This can probably be explained as follows as compared to the profound aversive effect of LiCl, APO and $CuSO_4$ induce a less pronounced CTA, taking also in account the high variability in the aversive response to the UCS from rat to rat, the possibility increases that a significant aversive effect of the UCS is no longer obtained The variability in the aversive response corresponds to the literature data [7,18] and necessitates including an aversive and a non-aversive control group in each series of experiments

Antiaversive Effect of the Opioids

To study the antiaversive effect of the opioids, these compounds were injected between the presentation of the CS and the administration of the UCS each time both stimuli were associated The influence of the opioids on the noxious or nauseous effect of the UCS (=emetics) is thus directly tested without interfering with the drinking behavior, the conditioning process is thus prevented by blocking the effect of the UCS This method is preferable to other methods such as the antagonism of an already established CTA [7,10]

From the seven opioids tested only three compounds, each at only one dose, showed a reproducible antiaversive effect against only one UCS the μ-agonist morphine (3 mg/kg) and the κ-agonist EKC (0 1 mg/kg) against APO and the δ-agonist DALA (0 1 mg/kg) against LiCl An opposite effect was observed with the higher doses of bremazocine and DALA these compounds potentiated the aversive effect of APO and LiCl, and of $CuSO_4$ respectively From these results the conclusion can be drawn that there is no parallelism between the effect of opioids against the different UCS and that agonists, selective for a particular opioid receptor type, can have both antiaversive and potentiating effects

The observation that naloxone antagonizes the antiaversive effect of morphine and EKC seems to point to the involvement of an opioid receptor mediated mechanism for their antiaversive effect, one has however to be cautious because the opioid antagonist naloxone has also non specific actions [25] Although the antiaversive effect of some opioids is naloxone sensitive, the opioid antiaversive effect cannot be attributed to interaction with a particular opioid receptor type, as was shown for their antiemetic effect in the dog [6] We consider this as a first argument against the use of the CTA test as a screening test for antiemetic drugs A second argument is the potentiating effect of some opioids on the aversion of the UCS As for the antiaversive effect, this potentiating effect was not related to a particular receptor type in contrast to the emetic effect of opioids in the dog [12] A potentiating effect can be considered as an addition of an aversive effect of the opioid to the aversive effect of the UCS The potentiating doses of bremazocine and DALA were therefore tested for a possible aversive effect by using them as UCS We did however not succeed in demonstrating a consistent aversive effect of these agents, no more as we did for morphine (3, 15 and 30 mg/kg) (unpublished data) The lack of a consistent aversive effect of morphine in our experiments is in contrast with earlier findings showing that morphine in doses from 10 mg/kg on can induce CTA [8,16] An explanation for this discrepancy can probably be found in the different experimental set-ups and statistical analyses of these various CTA studies Furthermore, the fact that the aversive effect of morphine is partially [19,27] or not at all [2] influenced by naloxone points to a largely aspecific mechanism for this effect of morphine This is again in contrast with the emetic effect of morphine [12] and of the δ-agonists [Met^5]enkephalin [3] and [Leu^5]enkephalin [9] which can be completely blocked by naloxone

Because neither the antiaversive nor the aversive effects of the opioids within the experimental conditions used are comparable to their antiemetic or emetic properties, we conclude that the CTA test cannot replace the emesis test for the study of antiemetics These findings also again shed doubt on the nausea concept in the CTA paradigm as was already reported [14]

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