Pharmacology Biochemistry & Behavior, Vol. 25, pp. 595–598, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Potentiation of the Propunishment, but not the Convulsant Action of the β-Carboline DMCM by Naltrexone

T. DUKA[1] AND D. N. STEPHENS

Research Laboratories of Schering AG, Berlin-West/Bergkamen, FRG

Received 29 January 1986

DUKA, T. AND D. N. STEPHENS. *Potentiation of the propunishment, but not the convulsant action of the β-carboline DMCM by naltrexone.* PHARMACOL BIOCHEM BEHAV **25**(3) 595–598, 1986.—The ability of naltrexone (NTX) to potentiate the propunishment and convulsant properties of DMCM, a benzodiazepine receptor inverse agonist, was studied in mice. Doses (0.39 and 1.56 mg/kg) of DMCM which were below the threshold for propunishment effects showed a marked ability to enhance the suppressive effects of punishment on locomotor activity in the presence of naltrexone (0.5 or 2.5 mg/kg IP), higher doses of DMCM and NTX (3.13 mg/kg and 10 mg/kg, respectively) had a depressant effect of their own on both punished and unpunished locomotor activity. DMCM given alone induced clonic convulsions (ED_{50}: 5.7 mg/kg IP) but this activity was not changed in the presence of naltrexone. These results suggest an interaction of BZ receptors and opioid systems in the control of anxiety.

DMCM Naltrexone Anxiety Convulsions Mice Benzodiazepine Receptor

IT is generally agreed that the benzodiazepines (BZs) produce their pharmacological effects by binding to receptor sites which modulate the activity of GABA [5, 10, 15]. By this primary mechanism of action, other neurotransmitter systems are affected and mediate the expression of the BZs' various effects. Several reports suggest a functional relationship between BZs and opiates. Although certain BZ properties, including their sedative effects [20] and discriminative stimulus properties [28] appear to be unrelated to endorphins, others, including their ability to induce hyperphagia [9], to enhance rates of self-stimulation [22], to induce ataxia [14] and to increase locomotor activity [7] are antagonised by the broad spectrum opiate antagonists naloxone or naltrexone.

Of particular interest is the interaction of BZ and opiate systems in conflict behaviour and other anxiety models. Both the BZs, and in certain cases opiates, possess significant anxiolytic properties [16,23], and additionally naloxone markedly reduces the disinhibitory properties of BZs in conflict tests at doses specific for opiate receptor antagonism [13, 19, 27], although these observations have not always been confirmed [6].

Recently, substances have become available which, although acting via the BZ receptor, exert pharmacological effects opposite to those of the BZs [4] Among these the β-carboline DMCM, 6,7-dimethoxy, 4-ethyl β-carboline carboxylic acid methyl ester, is the most potent. DMCM is convulsant [24] and at lower doses exerts a marked anxiogenic activity in several animal models [25, 29, 30]. We were therefore interested in discovering the nature of the interaction between an opiate antagonist and DMCM in its anxiogenic and convulsant activity. The opiate antagonist chosen was naltrexone (NTX) which is more potent and shows a longer half-life than naloxone [1].

METHOD

A modification of the four-plate test [2] was used, in which DMCM exhibits a propunishment effect at doses of 3 mg/kg and above [30]. Naive NMRI mice of either sex, supplied by the Department Tierzucht und -haltung, Schering AG, and weighing 25±2 g were placed individually in the centre of a rectangular chamber (23×18×30 cm high) whose floor was divided into four equally sized metal plates. Following 20 sec in which the mouse was allowed to explore freely, it received a mild (0.3 mA) and brief (60 msec) electric shock each time it crossed from one plate to another. The number of such crossings in a 1-min period were taken as a measure of exploratory activity. As a control for sedative or stimulant effects, the effect of test substances on non-punished crossings was assessed in independent groups. In both punished and unpunished conditions eight mice were tested per drug dose.

[1]Requests for reprints should be addressed to Dr. T. Duka, Department of Neuroendocrinology and Neuropsychopharmacology, Schering AG, Postfach 65 0311, D-1000 Berlin 65.

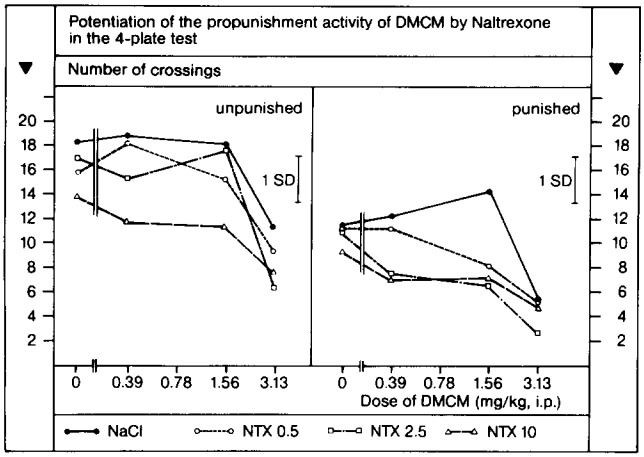

FIG. 1. Number of crossings in the four plate apparatus in unpunished or punished group of mice (footshock, 0.3 mA, 60 msec), following different combinations of treatments of DMCM and naltrexone.

TABLE 1

NUMBER OF MICE OUT OF 8 SHOWING CLONIC CONVULSIONS

DMCM Dose (mg/kg, IP)	Naltrexone Dose (mg/kg, IP) 0	0.5	2.5
0	0	0	0
1.56	0	0	0
3.13	1	0	0
6.25	4	5	6
12.5	8	6	7
ED_{50}	5.7	7.6	6.4
95% confidence limits	(4.0–8.1)	(5.1–12.9)	(4.4–9.3)

Effect of naltrexone 0.5 mg/kg and 2.5 mg/kg on DMCM induced clonic convulsions. Naltrexone was injected IP 20 min before DMCM. DMCM was injected IP and the number of mice showing clonic convulsions was noted. ED_{50}s were estimated by the method of Litchfield and Wilcoxon [21].

Drugs were injected 20 min before testing in a 4 (saline, 0.5 mg/kg and 2.5 mg/kg and 10 mg/kg naltrexone) × 4 (cremophor vehicle, 0.39 mg/kg, 1.56 mg/kg and 3.13 mg/kg DMCM) design.

DMCM doses were chosen to include doses both below and above the minimally effective dose for propunishment activity [30]. In a separate experiment 1 mA instead of 0.3 mA electric shock was applied, and cremophor vehicle, 0.5 mg/kg or 2.5 mg/kg NTX were administered. Testing was performed as described above.

The ability of the opiate antagonist to enhance the convulsant activity of DMCM was also tested. Saline or naltrexone (0.5 or 2.5 mg/kg) were administered IP and 20 min later DMCM administered, also IP, in the dose 1.56, 3.13, 6.25 or 12.5 mg/kg to groups of eight mice. The number of mice exhibiting clonic seizures during the following 15 minutes was noted.

STATISTICS

Analysis of variance of two factors (DMCM-dose and NTX-dose) was performed for unpunished or punished locomotor activity in the 4-plate procedure. Data are presented as means, and a significant effect is considered to have occurred when $p<0.05$.

RESULTS

Figure 1 illustrates that both DMCM, $F(3,112)=32.0$, $p<0.001$, and NTX, $F(3,112)=9.8$, $p<0.001$, reduced unpunished activity and that in each case this effect was attributable to the highest doses of the two substances (Scheffé, $p<0.01$). There was no significant interaction ($F(9,112)=1.54$; NS) in the effects of NTX and DMCM.

Punishment resulted in a reduction of locomotor activity which was potentiated in the presence of DMCM, $F(3,112)=22.8$, $p<0.001$. Post hoc analysis indicated this effect to be due to the highest (3.13 mg/kg) dose of DMCM. Naltrexone also enhanced the effects of punishment, $F(3,112)=11.3$, $p<0.001$, again as a result of the highest NTX dose (10 mg/kg), the lower doses showing no effect. A significant interaction term, $F(9,112)=3.4$, $p<0.001$, indicates that the propunishment effects of NTX and DMCM potentiated each other. As can be seen from Table 2, NTX on its own did not show any propunishment effect at a higher level of footshock, 1 mA.

Table 1 indicates that DMCM induced seizures in a dose-dependent fashion and that this relationship was not significantly altered by either 0.5 or 2.5 mg/kg naltrexone.

DISCUSSION

The present study demonstrates a potentiation of the propunishment action of subthreshold doses of DMCM by the opiate antagonist naltrexone. This interaction between the opiate antagonist and the inverse agonist at benzodiazepine receptor is consistent with the ability of another opiate antagonist, naloxone, to antagonise the anxiolytic activity of BZ agonists [13, 19, 27] and suggests that opiate and GABAergic system in the brain may interact in the control of anxiety or stress reactions. Furthermore, this interaction seems to be specific for anxiety, since the ability of NTX to potentiate the action of DMCM was limited to DMCM's anxiogenic action and did not extend to its convulsant activity.

Although the data indicating a potentiation of DMCM's anxiogenic activity by NTX are clear in this test, the basis for such an interaction is by no means certain. Since DMCM does not bind to opioid receptors [4] and naltrexone does not bind to BZ receptors [3] a direct interaction at the level of the receptor can be ruled out. Some evidence suggests, however, that BZs can induce the release of opioids [11,32] and in common with their anxiolytic, sedative and anticonvulsant activity this effect of BZs appears to be mediated by GABA, since it is antagonised by bicuculine and mimicked by muscimol and the GABA-transaminase inhibitor aminooxyacetic acid [12]. Since DMCM acts via BZ receptors to reduce the effectiveness of GABA [4] it seems possible that DMCM would reduce opioid release, and this might provide a basis for the additive effects of DMCM and NTX in potentiating the effect of punishment. However, it seems unlikely that the anxiolytic effects of BZs and anxiogenic actions of BZ receptor inverse agonists depend entirely on their ability to modulate opioid release.

TABLE 2

NUMBER OF CROSSINGS (MEAN ± SEM), IN THE FOUR PLATE APPARATUS IN UNPUNISHED OR PUNISHED GROUP OF MICE (FOOT SHOCK 1 mA, 60 msec) FOLLOWING DIFFERENT NALTREXONE DOSES

	NaCl	Naltrexone 0.5 mg/kg	Naltrexone 2.5 mg/kg
Unpunished crossings	13.3 ± 0.9	13.5 ± 1.2	10.6 ± 0.8
Punished crossings	5.9 ± 1.0	6.4 ± 0.8	6.3 ± 1.0

In seeking alternative explanations, effects of NTX on pain threshold and on locomotor activity can be excluded. The possibility that NTX decreased pain threshold and thus intensified the punishing properties of shock seems unlikely for two reasons: firstly, at the lower doses used, naltrexone on its own had no propunishment effects but still potentiated the effects of DMCM, and, secondly, opioid analgesics appear to have no specific anti-punishment properties in the four-plate test ([2] and unpublished).

The failure of low doses of either DMCM or naltrexone, or the combination of low doses to influence unpunished locomotor activity in the novel environment would also seem to rule out explanations based on naltrexone's ability to reduce exploratory activity [18], at least at these low doses.

The highest dose of naltrexone (10 mg/kg) which may not be specific for opiate receptors induced a decrease in both punished and unpunished locomotor activity. In this case the ability of high doses of NTX to reduce exploratory activity [8,18] may have influenced the number of punished crossings. On the other hand, since DMCM also decreased unpunished exploratory activity at the highest dose (Fig. 1), we assume that a strong anxiogenic effect induced by the substances themselves, may result in behavioural inhibition and influence exploratory activity even in the absence of punishment.

Another mechanism in which DMCM and naltrexone would be expected to act synergistically is in the physiological response to stress. If in the presence of DMCM the footshock (0.3 mA for 60 msec) became stressful then opioid release would be expected to take place [26]. Alternatively, the doses of DMCM used may have been sufficiently stressful in themselves to induce opioid release, since we have previously shown that DMCM potently induces one stress response, an increase in plasma corticosterone levels [31].

In either case, naltrexone might act to antagonise the ability of the released opioids to counteract the stress and thus intensify the effects of punishment. At a higher level of foothshock, 1 mA, which would itself be expected to be stressful, naltrexone did not show any propunishment effect (Table 2), but the activity levels were already very low in these animals.

Finally, it cannot be excluded that naltrexone by itself or through the blockade of opioid peptide transmission, may alter nonopiate processes involved in the propunishment (anxiogenic) activity of DMCM. For instance an interaction has been demonstrated between DMCM and the noradrenergic system with respect to the anxiogenic effect of DMCM [31], and an interaction between opioids and noradrenergic systems has been suggested [17].

Whatever the mechanisms, the present findings that the anxiogenic activity of subthreshold doses of a BZ receptor inverse agonist is potentiated by an opioid antagonist provide further evidence of an interaction between BZ receptors and opioid systems in the control of punished behaviour, consistent with previous findings of an antagonism of the anxiolytic properties of BZ receptor agonists by opioid antagonists.

ACKNOWLEDGEMENTS

We thank Ronald Weidmann for his skilled assistance, Dr. A. van der Linde for statistical advice, A. Dahrmann for typing the manuscript and Dr. R. Jacobsen, Endo Laboratories for the gift of Naltrexone.

REFERENCES

1. Blumberg, H. and H. B. Dayton. Naloxone, naltrexone, and related noroxymorphones. In: *Narcotic Antagonists. Advances in Biochemical Psychopharmacology*, vol 8, edited by M. C. Braude, L. S. Harris, E. L. May, J. P. Smith and J. E. Villareal. New York: Raven Press, 1974, pp. 33–43.
2. Boissier, J. R., P. Simon and C. Aron. A new method for rapid screening of minor tranquillizers in mice. *Eur J Pharmacol* **4:** 145–150, 1968.
3. Braestrup, C. and R. F. Squires. Specific benzodiazepine receptors in rat brain characterized by high-affinity (^{3}H)diazepam binding. *Proc Natl Acad Sci USA* **74:** 3805–3809, 1977.
4. Braestrup, C., R. Schmiechen, G. Neff, M. Nielsen and E. N. Petersen. Interaction of convulsive ligands with benzodiazepine receptors. *Science* **216:** 1241–1243, 1982.
5. Braestrup, C. and M. Nielsen. Benzodiazepine receptors. In: *Handbook of Psychopharmacology*, vol 17, edited by L. L. Iversen, S. D. Iversen and S. H. Snyder. New York: Plenum, 1983, pp. 285–384.
6. Britton, D. R., K. Thatcher Britton, D. Dalton and W. Vale. Effects of naloxone on anti-conflict and hyperphagic actions of diazepam. *Life Sci* **29:** 1297–1302, 1981.
7. Castellano, C., U. Filibeck and F. Pavone. Naltrexone-reversible effects of flunitrazepam on locomotor activity and passive avoidance behaviour in mice. *Eur J Pharmacol* **104:** 111–116, 1984.
8. Castellano, C. and S. Puglisi-Allegra. Effects of naloxone and naltrexone on locomotor activity in C57BL/6 and DBA/2 mice. *Pharmacol Biochem Behav* **16:** 561–563, 1982.
9. Cooper, S. J. Benzodiazepine-opiate antagonist interactions and reward processes: implications for drug dependency. *Neuropharmacology* **22:** 535–538, 1983.
10. Costa, E. and A. Guidotti. Molecular mechanisms in the receptor action of benzodiazepines. *Annu Rev Pharmacol Toxicol* **19:** 531–535, 1979.
11. Duka, T., M. Wüster and A. Herz. Rapid changes in enkephalin levels in rat striatum and hypothalamus induced by diazepam. *Naunyn Schmiedebergs Arch Pharmacol* **309:** 1–5, 1979.
12. Duka, T., M. Wüster and A. Herz. Benzodiazepines modulate striatal enkephalin level via a GABAergic mechanism. *Life Sci* **26:** 771–776, 1980.
13. Duka, T., R. Cumin W. Haefely and A. Herz. Naloxone blocks the effect of diazepam and meprobamate on conflict behaviour in rats. *Pharmacol Biochem Behav* **15:** 115–117, 1980.

14. File, S. E. Chlordiazepoxide-induced ataxia, muscle relaxation and sedation in the rat: effect of muscimol, picrotoxin and naloxone. *Pharmacol Biochem Behav* **17:** 1165–1170, 1982.
15. Haefely, W., A. Kulcsar, H. Möhler, L. Pieri, P. Polc and R. Schaffner. Possible involvement of GABA in the central actions of benzodiazepines. In: *Mechanism of Action of Benzodiazepines*, edited by E. Costa and P. Greengard. New York: Raven Press, 1975, pp. 131–151.
16. Jarvik, L. F., J. H. Simpson, D. Guthrie and E. H. Liston. Morphine, experimental pain, and psychological reactions. *Psychopharmacology (Berlin)* **75:** 124–131, 1981.
17. Khachaturian, H. and S. J. Watson. Some perspectives on monoamine-opioid peptide interaction in rat central nervous system. *Brain Res Bull* **9:** 441–462, 1982.
18. Koek, W. and J. L. Slangen. Acute effects of naloxone and naltrexone, but lack of delayed effects, on exploratory behavior in the rat. *Psychopharmacology (Berlin)* **84:** 383–387, 1984.
19. Koob, G. F., R. E. Strecker and F. E. Bloom. Effects of naloxone on the anticonflict properties of alcohol and chlordiazepoxide. *Subst Alcohol Actions Misuse* **1:** 447–457, 1980.
20. Kraynack, B., J. Gintautas, T. Hughston and D. DeShan. Failure of naloxone to antagonize diazepam induced narcosis in rats. *Proc West Pharmacol Soc* **24:** 189–190, 1981.
21. Litchfield, J. T. and F. Wilcoxon. A simplified method of evaluating dose-effect experiments. *J Pharmacol Exp Ther* **96:** 99–104, 1949.
22. Lorens, S. A. and S. M. Sainati. Naloxone blocks the excitatory effect of ethanol and chlordiazepoxide on lateral hypothalamic self-stimulation behavior. *Life Sci* **23:** 1359–1364, 1978.
23. Millan, M. J. and T. Duka. Anxiolytic properties of opioids and endogenous opioid peptides and their relationship to the actions of benzodiazepines. *Med Probl Pharmacopsychiatry* **17:** 123–141, 1981.
24. Petersen, E. N. DMCM: A potent convulsive benzodiazepine receptor ligand. *Eur J Pharmacol* **94:** 117–124, 1983.
25. Petersen, E. N. and L. H. Jensen. Proconflict effect of benzodiazepine receptor inverse agonists and other inhibitors of GABA function. *Eur J Pharmacol* **103:** 91–97, 1984.
26. Rossier, J., E. French, C. Rivier, N. Ling, R. Guillemin and F. E. Bloom. Footshock-induced stress increases beta-endorphin levels in blood but not in brain. *Nature* **270:** 618–620, 1977.
27. Soubrié, P., A. Jobert and M. H. Thiebot. Differential effects of naloxone against the diazepam-induced release of behavior in rats in three aversive situations. *Psychopharmacology (Berlin)* **69:** 101–105, 1980.
28. Shearman, G. T., M. J. Millan and A. Herz. Lack of evidence for a role of endorphinergic mechanisms in mediating a discriminative stimulus produced by diazepam in rats. *Psychopharmacology (Berlin)* **78:** 282–284, 1982.
29. Stephens, D. N., G. T. Shearman and W. Kehr. Discriminative stimulus properties of β-carbolines characterized as agonists and inverse agonists at central benzodiazepine receptors. *Psychopharmacology (Berlin)* **83:** 233–239, 1984.
30. Stephens, D. N. and W. Kehr. β-Carbolines can enhance or antagonize the effects of punishment in mice. *Psychopharmacology (Berlin)* **85:** 143–147, 1985.
31. Stephens, D. N., W. Kehr and T. Duka. Anxiolytic and anxiogenic β-carbolines: tools for the study of anxiety mechanisms. In: *Gabaergic Transmission and Anxiety. Advances in Biochemical Psychopharmacology*, edited by G. Biggio and E. Costa. New York: Raven Press, 1986.
32. Wüster, M., T. Duka and A. Herz. Diazepam-induced release of opioid activity in the rat brain. *Neurosci Lett* **16:** 335–337, 1980.