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An Anxiolytic-Like Effect of Ondansetron Disappears in Oxazepam-Tolerant Rats

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NOWAKOWSKA, E., A. CHODERA AND K. KUS. *An anxiolytic-like effect of ondansetron disappears in oxazepam-tolerant rats.* PHARMACOL BIOCHEM BEHAV **59**(4) 935–938, 1998.—In our experiments a drug from the group of 5-HT_3 antagonists—ondansetron (OND)—has been used in rats developing tolerance to oxazepam (OXZ). After 7 days of oxazepam administration (5 mg/kg IP) a significant decrease in the anxiolytic behavior was observed in the Crawley test. In the rats already partly tolerant to oxazepam, an undiminished anxiolytic-like effect of ondansetron (single injection of 0.1 mg/kg IP, seventh day) was observed. After 14 days of oxazepam administration its anxiolytic activity was even more diminished. A single injection of ondansetron 0.1 mg/kg restored the anxiolytic behavior: rise of BWT (black–white transition) and WSE (white square entrance). After 21 days the anxiolytic activity of oxazepam was totally abolished and the single injection of ondansetron did not restore the state of anxiolysis. The results show that the anxiolytic effects of ondansetron were not influenced in the first stages of tolerance development to oxazepam, but the drug was not able to produce an anxiolytic effect in the state of full tolerance to oxazepam (after 3 weeks). ©1998 Elsevier Science Inc.

Anxiolytic effect Tolerance Oxazepam 5-HT_3 antagonist ondansetron

BENZODIAZEPINES are a group of drugs that are most frequently used as anxiolytics. The therapeutic problems connected with benzodiazepine use are: 1) diminished activity after long treatment, and 2) withdrawal effects (anxiety, sleeplessness, myospasms, or even seizures). Those are the reasons that warrant looking for drugs, which can substitute benzodiazepines and/or are able to abolish signs of tolerance and withdrawal.

One of these drug groups are the 5-HT_3 receptor antagonists. It was shown by Costall et al. (2), that the 5-HT_3 antagonist zacopride was in some experiments about 100 times more potent than diazepam. Some other 5-HT_3 receptor antagonists, however, showed little anxiolytic effect (6). Until now, however, mainly conflicting evidence regarding the efficacy of 5-HT_3 receptor antagonists in benzodiazepine withdrawal have been obtained (11). Although in one test (the elevated plus-maze) ondansetron (OND) completely reversed the effect of flumazenil, it failed to block the flumazenil action in other tests. Goudie et al. (7) showed, that ondansetron attenuated benzodiazepine withdrawal, in a U-shaped response curve, 0.1 mg/kg being the active dose. No data are available concerning 5-HT antagonist/benzodiazepine interaction during the development of tolerance.

The aim of our experiments was to learn, if in the state of tolerance to the anxiolytic activity of a benzodiazepine ($GABA_A$ receptor activator) a drug acting through another mechanism—a 5-HT_3 receptor antagonist can still exert its anxiolytic effect. Ondansetron and oxazepam (OXZ) were chosen, because there is no known interaction between these drugs and also because their half-life time is very similar, about 3–6 h (5,14).

METHOD

Animals

Male Wistar rats (200 ± 20 g) aged 10–12 weeks, bought from a breeder (license of the Ministry of Agriculture) were used in the study. The animals were housed in standard laboratory conditions under a 12 L:12 D cycle (lights on at 0600 h), in a temperature-controlled room 21 ± 2°C, humidity 70%, with free access to granulated standard food and tap water. The rats were kept four per cage (30 × 30 × 20 cm). Each experimental and control group consisted of eight animals.

Drugs

Oxazepam (OXZ) substance from Polfa Poznaá; carboxymethylcellulose (CMC) from BDI, UK; Ondansetron

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(inj.) (OND) from (GR 380 32F) from Glaxo, UK. Oxazepam was suspended in 0.5% solution of CMC and administered intraperitoneally (IP) 25 min before test. OND was injected also IP, 60 min before the test. CMC was administered IP in control animals 60 and 25 min before the test. The injected volume of drug solutions and CMC were always 0.5 ml.

Method

Anxiolytic effects were determined according to the two compartment exploratory test of Crawley (3), in the modification of Merlo-Pich and Samanin (10). Animals were assigned randomly to treatment and vehicle control groups. Each of the rats received its treatment IP 60 and 25 min before being placed into the modified open field. In the field there is a dark (black), covered area, divided into four squares and an illuminated (white) area of 21 squares. All rats were placed gently in the same peripheral lit square close to the black compartment at the start of the session. Separate counts of movements in the dark area (black square entrances = BSE) and the illuminated area (white square entrances = WSE) were recorded as well as the number of transitions between compartments (black–white transitions = BWT). The records were performed by an observer sitting quietly 2 meters away, unaware of the treatment. The rats were allowed to move freely between the squares and the compartments. Whenever an animal crossed the border line between two squares or the area transition line with all four legs, an event was recorded. Each rat was tested only once daily, always between 1000–1300 h.

Locomotor activity test was performed to determine the dose, which, having a distinct anxiolytic effect, has no influence on locomotor activity. For this purpose a PAN licence Activity Meter was used, the locomotor activity was counted automatically for 5 min.

Single Dose Procedure

Experiments were performed, using varying doses of drugs OXZ 1.0, 2.5, 5.0, and 10.0 mg/kg, OND 0.01, 0.1, and 0.3 mg/kg.

Chronic Experiment Procedure

In the chronic experiments three group of rats were investigated simultaneously. Two groups received an injection of 5 mg/kg OXZ IP once daily, and were investigated in Crawley's test for anxiolytic activity after 7, 14, and 21 days. One group received (only on test day) ondansetron 0.1 mg/kg IP 60 min before the test. The reference group (OXZ alone) received instead of ondansetron a vehicle injection. The control group (without drugs) received, instead of the drugs, vehicle injections.

The animals were investigated in the two-compartment exploratory test of Crawley (2) in modification of Merlo-Pich and Samanin (10), as described above.

Statistical Analysis

The data are shown as means ± SEM. Statistical analysis was performed using two-way ANOVA followed by Student's *t*-test (13) for dependent samples and using Friedman test. The confidence limit of $p < 0.05$ was considered statistically significant.

RESULTS

Single Dose Experiments

In Table 1 the results of experiments with different single doses of OXZ and OND are shown. The control animals showed a distinct preference for the dark compartment (BSE = 9.5 ± 1.2), they trespassed the borderline to the white compartment very seldom (BWT = 2.6 ± 0.5), and they did not move much in the white compartment (WSE = 1.2 ± 0.4).

TABLE 1

THE EFFECT OF OXAZEPAM AND ONDANSETRON ON BSE, BWT, AND WSE PARAMETERS IN THE TWO-COMPARTMENT TEST IN RATS (n = 8, IN EACH GROUP)

Drug	Doses (mg/kg)	BSE (mean ± SEM)	BWT (mean ± SEM)	WSE (mean ± SEM)
Control (CMC, IP)		9.5 ± 1.2	2.6 ± 0.5	1.2 ± 0.4
Oxazepam IP	1.0	8.4 ± 1.1	2.7 ± 0.3	1.1 ± 0.7
	2.5	10.2 ± 1.8	12.5* ± 3.1	24.9* ± 5.4
			(F = 9.9)	(F = 19.2)
	5.0	14.2* ± 2.1	12.1* ± 2.1	24.1* ± 3.9
		(F = 3.8†)	(F =19.4)	(F = 34.1)
	10.0	19.9* ± 1.7	16.1* ± 1.1	27.9* ± 2.5
		(F = 25.0)	(F = 124.8)	(F = 111.2)
Ondansetron IP	0.01	9.9 ± 1.1	2.1 ± 0.3	1.5 ± 0.4
	0.10	11.4 ± 1.9	6.7* ± 1.1	15.2* ± 1.2
			(F = 11.5)	(F = 122.5)
	0.30	12.5 ± 2.9	8.1* ± 0.3	18.3* ± 4.1
			(F = 89.0)	(F = 17.2)

BSE—black square entrance
WSE—white square entrance
BWT—black-white transition
OND was given 60 min and OXZ 25 min before the test.
*Difference statistically significant ($p < 0.05$) in comparison with the control group.
†$p < 0.1$.

TABLE 2

THE EFFECT OF ONDANSETRON IN RATS DEVELOPING TOLERANCE TO OXAZEPAM (TWO-COMPARTMENT TEST)

Drugs (mg/kg)	BSE (mean ± SEM)	BWT (mean ± SEM)	WSE (mean ± SEM)
A. Control (CMC, IP)	9.5 ± 1.2	2.6 ± 0.5	1.2 ± 0.3
B. Oxazepam single admin.	14.2† ± 2.1 (F = 3.8*)	13.1† ± 2.1 (F = 23.7)	24.9‡ ± 2.1 (F = 124.8)
C. Ondansetron single admin.	11.4 ± 1.9	6.7 ± 1.1	15.4† ± 1.9
A′. Control CMC 7 days	9.1 ± 1.1	2.3 ± 0.3	1.1 ± 0.2
D. Oxazepam 7 days	13.5 ± 2.4	6.2‡ ± 1.4 (F = 5.1)	12.5‡ ± 3.9 (F = 7.8)
E. Oxazepam 7 days + single admin. of ondansetron	15.4 ± 4.2	14.7 ± 4.1	29.8§ ± 5.9 (F = 6.0)
A′′. Control CMC 14 days	9.4 ± 1.1	2.1 ± 0.3	1.5 ± 0.5
F. Oxazepam 14 days	10.5 ± 2.1	4.2‡ ± 1.2 (F = 13.5)	2.6‡ ± 0.3 (F = 110.5)
G. Oxazepam 14 days + single admin. of ondansetron	12.4 ± 1.5	19.6† ± 4.2 (F = 8.8)	28.4‡ ± 4.1 (F = 8.3)
A′′′. Control CMC 21 days	9.1 ± 1.5	2.5 ± 0.3	1.8 ± 0.6
H. Oxazepam 21 days	10.9 ± 1.1	3.4# ± 1.2 (F = 16.1)	0.3# ± 0.1 (F = 136.9)
I. Oxazepam 21 days + single admin. of ondansetron	8.5 ± 2.8	4.4 ± 2.1	5.3# ± 2.3 (F = 11.5)

Dosage: oxazepam 5 mg/kg, ondansetron 0.1 mg/kg, (n = 8 in each group).

BSE—black square entrance; WSE—white square entrance, BWT—black–white transition; OND was given 60 min and OXZ 25 min before the test.

*$p < 0.1$.

†Statistically significant difference ($p < 0.05$) for B, C vs. A.

‡Statistically significant difference ($p < 0.05$) for D, F vs. B.

§Statistically significant difference ($p < 0.05$) for E vs. D.

¶Statistically significant difference ($p < 0.05$) for G vs. F and C.

#Statistically significant difference ($p < 0.05$) for H, I vs. B and C.

The administration of drugs caused a significant increase of all three parameters after 5 and 10 mg of OXZ, while after OND 0.1 and 0.3 mg/kg only BWT and WSE were significantly increased. For the chronic experiments the doses of 5.0 mg/kg OXZ and 0.1 mg/kg OND were chosen, because, having a distinct anxiolytic effect, they did not show influence on the locomotor activity of rats (separate experiments). The 5-min counts in the Activity Meter were: for oxazepam 5 mg/kg 95.8 ± 11.2, for ondansetron 0.1 mg/kg 83.9 ± 10.8 against control values (vehicle application) 94.6 ± 12.5.

Chronic Experiments

As shown in Table 2, after 7 days of oxazepam administration a significant decrease of the anxiolytic behavior was observed in BWT and WSE (group D). After additional injection of OND, however, the initial anxiolytic activity was restored or even surpassed (E group).

After 14 days of oxazepam administration its anxiolytic activity was almost abolished—a strong decrease of all parameters with the exception of the BSE parameter occurred (group F). A single injection of 0.1 mg/kg of ondansetron restored the anxiolytic behavior (rise of BWT and WSE)—group G.

After 21 days of oxazepam administration the anxiolytic activity of OXZ was totally abolished and the single injection of ondansetron did not restore the state of anxiolysis, the values of BWT and WSE were significantly lower than in the reference groups (group H and I vs. B and C). The control animals, receiving only vehicle through 21 days and retested after 7, 14, and 21 days (groups A, A′, A′′, and A′′′), showed no change of observed parameters BSE, BWT, and WSE (Table 2).

DISCUSSION

The experimental results show that after 7 and 14 days of OXZ treatment the OXZ anxiolytic effects disappeared, but the OND effect is not only present but even increased (additive synergism of OXZ and OND). This may indicate that the pathways for tolerance are at least partially different. The disappearance of OND effect in deeper tolerance (after 3 weeks of OXZ treatment) seems, however, to indicate that after a long enough treatment with the benzodiazepine, a factor may come to life (8), which causes the loss of anxiolytic activity of ondansetron. This is similar to the locomotor activity tests with benzodiazepines, in which after chronic treatment, these initially sedating drugs become locomotor stimulants (1,12).

It ought to be said that in earlier experiments (unpublished data) after ondansetron being administrated once a week, with every day application of CMC, no signs of tolerance to the drug occurred.

We suppose that the tolerance to the anxiolytic and sedative effects of benzodiazepines may be due to receptor down-regulation (12), whereas the loss of anxiolytic activity and the reported locomotor stimulation effect (1) cannot be explained in this way. The above-mentioned symptoms may be part of the withdrawal syndrome to OXZ. The formation of an anti-

anxiolytic factor, which could not be overcome by joint administration of OXZ and OND in the test is one of the possible explanations. The link between tolerance and withdrawal was observed also by other authors (4). In connection with our results, it may be worth mentioning that in the study with chronically administered chlordiazepoxide, reported by Leathley (9), the 5-HT_3 antagonist tropisetron (ICS 205-930) did not show any effect alleviating the withdrawal symptoms.

The conclusion is, that after reaching the state of full tolerance to benzodiazepines, a drug with another action mechanism (a 5-HT antagonist) is not able to exert an anxiolytic effect.

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