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INFLUENCE OF CCK RECEPTOR AGONIST AND ANTAGONISTS ON TOLERANCE INDUCED TO ANTI-PENTYLENETETRAZOL ACTIVITY OF DIAZEPAM

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ABSTRACT

In the present study, the effects of caerulein and CCK receptor antagonists on tolerance to the anti-pentylenetetrazol activity of diazepam has been studied. Different doses of diazepam (20, 30, 35, 40 and 50 mg/kg) were administered intraperitoneally for a period of 6 days in order to induce tolerance to diazepam. 48 h after the last dose of diazepam, a test dose of diazepam was tested for anti-pentylenetetrazol activity. When animals were treated with caerulein or the CCK receptor antagonists MK-329 and L-365, 260, only L-365, 260 reversed the tolerance to diazepam.

It may be concluded that CCK-B receptor mechanisms may interact with tolerance induced by diazepam.

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INTRODUCTION

The potent and broad anticonvulsant activity of benzodiazepine agonists leads to many therapeutic applications, including petit mal and status epilepticus.[13] Benzodiazepine anxiolytics exert their pharmacological effects through specific recognition sites on the γ-aminobutyric acid (GABA) receptor complex.[27] Decrease in sensitivity to the sedative,[12] muscle relaxant,[18] anxiolytic and anticonvulsant effects of benzodiazepines have been reported.

Diazepam, clonazapam, clobazam and nitrazepam are therapeutically administered as antiepileptics.[19,20] For chronic treatment of epilepsies, benzodiazepine receptor agonists are not the first choice (in spite of a much better tolerability than other anti-epileptics), except for certain forms of epilepsy in children. The main reason for the restricted use of benzodiazepines is the development of tolerance in a considerable proportion of patients.[11,28] It is also observed that up-regulation of sulphated [^{3}H]CCK-8 binding can be found after chronic diazepam administration.[7]

In the present work, the effects of CCK receptor agonist and antagonists on diazepam-induced tolerance have been studied.

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MATERIALS AND METHODS

Animals

Male albino mice weighing 20-30 g were used in the experiments. The animals remained in groups of 10 in their cage under conditions of constant temperature (21±2°C) and light control. Animals had free access to food and water except during the experiments. Each animal was used only once.

Table I: Induction of tolerance to anti-pentylenetetrazol effect of diazepam.

Treatment (mg/kg; 6 days)	Induction of Ataxia	Ataxia Score	Induction of Seizures	Seizure Score
Vehicle 10 mL/kg	47.8±1.3	2±0.0	0.0±0.0	0.0±0.0
Diazepam: 10,15,20,25,30,35	54.7±2.3	2±0.0	60±20*	0.5±0.2
Diazepam: 20,25,30,35,40,45	62.5±3.5**	1.5±0.2*	35.6±13.6	0.6±0.3
Diazepam 20,30,35,40,45,50	66.5±5**	1.5±0.2*	102±9.9***	2±0.3***

Mice were injected with either vehicle (DMSO 80%) or diazepam intraperitoneally (IP) for a period of 6 days. On the test day each animal received a test dose of diazepam (5 mg/kg) 10 min before pentylenetetrazol (100 mg/kg, IP) administration. Each point is the mean±SEM of 10 animals. *p<0.05, **p<0.01 ***p<0.001 different from vehicle control group.

Induction of tolerance to diazepam and anti-pentylenetetrazol testing

Different doses of diazepam (20, 30, 35, 40, 45 and 50 mg/kg, for a period of 6 days respectively) were administered intraperitoneally (IP) in order to induce tolerance to the anti-pentylenetetrazol activity of diazepam. All testing was done 48 h after, at which time approximately 99% of active diazepam had been eliminated from the brain. Testing was achieved based on the method of Rosenberg et al.[21]

On the test day, a test dose of diazepam was employed. Nine minutes later, motor function was evaluated by observing the mice on a table-top, and assigning an ataxia rating of 0 (no observable drug effect), 1 (slight ataxia), 2 (clear ataxia, falling or stumbling side ways), 3 (unable to stand, drags trunk on the table-top), or 4 (loss of righting response). One minute later (ten min. after diazepam injection), 100 mg/kg pentylenetetrazol (PTZ), in a volume of 1 mL/kg, was injected IP. The nature of convulsive activity, and the time to onset of myoclonus, front leg clonus, generalized clonus involving all four legs, and tonus was recorded for 20 min. A seizure score was assigned to each rat according to the most severe convulsion observed: 0, no convulsion or tremors, face and ear twitches only; 1, myoclonic jerks; 2, front leg clonus, no loss of upright posture; 3, severe clonic seizure with loss of upright posture; 4, tonic-clonic seizure.

Drugs

The chemicals used were diazepam (Hoffmann LaRoche), pentylenetetrazol (Aldrich, England), L-365, 260, and MK-329 (Merck Sharp and Dohme, England). Caerulein was dissolved in saline, while other drugs were dissolved in DMSO (40%) and distilled water (60%). The drugs were injected intraperitoneally (IP) in a volume of 10 mL/kg.

Statistical analysis

Two-way ANOVA followed by Newman-Keuls' test were used for analysis of the data. Differences between means were considered statistically significant if p<0.05.

RESULTS

Induction of tolerance to diazepam

Several schedules were chosen to be sure that tolerance could be detected. It was shown that the administration of six doses of diazepam (20, 30, 35, 40, 45 and 50 mg/kg, intraperitoneally) for six days respectively can induce tolerance. To evaluate the degree of tolerance to diazepam, forty-eight hours after administration of the last dose of benzodiazepine, a test dose of diazepam (5 mg/kg, IP) and pentylenetetrazol (100 mg/kg) was used. Repeated doses of of the benzodiazepine induced tolerance; the ataxia score was reduced [one-way ANOVA; $F(3, 36)= 4.2, p<0.05$] and the degree of convulsion was increased [one way ANOVA; $F(3, 36)= 13.4, p<0.0001$] (Table I).

Effect of daily administration of caerulein or CCK receptor antagonists on development of tolerance to diazepam

Two-way ANOVA indicates that tolerance to anti-

Table II: Effect of caerulein and CCK antagonists on development of tolerance induced by daily administration of diazepam.

Drug Treatment (mg/kg; 6 days)		Induction of Ataxia	Ataxia Score	Induction of Seizure	Seizure Score
Vehicle 10 mL/kg		45.8±3.5	1.9±0.0	0.0±0.0	0.0±0.0
Caerulein	0.05	69.2±4.9	2±0.0	0.0±0.0	0.0±0.0
MK-239	0.05	70.5±5.1	1.4±0.2	64.2±3.7	0.5±0.2
L-365.260	0.05	71.3±4.4	1.5±2	17.5±11.3	0.3±0.2
L-365.260	0.5	85±8.4	1.8±0.2	0.0±0.0	0.0±0.0
L-365.260	1.0	80±7.3	1.8±0.2	0.0±0.0	0.0±0.0
Diazepam		75.8±4.5	1.5±0.9	102±9.9	2±0.3
Caerulein	0.05+Diazepam	89±7.8	1.5±0.2	205±37.8	2.4±0.46
MK-329	0.05+Diazepam	85±8.4	1.8±0.2	285±139.6	1.9±0.3
L-365.260	0.05+Diazepam	107.5±6.2**	1.5±0.2	570±202	1±0.3*
L-365.260	0.5+Diazepam	86.9±2.1	1.8±0.2	187.5±17.7	1.9±2.1
L-365.260	1.0+Diazepam	95±5.7	1.8±0.2	330±133.2	2.1±0.4

One group of animals was given vehicle, caerulein, MK-329 or L-365.260 intraperitoneally (IP) for a period of 6 days. The second group of animals was given (IP) caerulein 30 min, MK-329, or L-365.260 5 min before daily injection of diazepam for a period of 6 days. On the test day each animal received a test dose of diazepam (5 mg/kg) for ataxia evaluation or diazepam (5 mg/kg) 10 min before pentylenetetrazol (100 mg/kg, IP) administration for evaluation of the anticonvulsant effect of diazepam. Each point is the mean±SEM of 10 animals. *p<0.05, **p<0.01, different from respective control group.

pentylenetetrazol activity of diazepam [F(1, 56)= 95.9, p<0.0001] was induced in animals treated once a day with diazepam (20, 30, 35, 40, 45 and 50 mg/kg, IP). When animals were co-administered daily diazepam with caerulein or CCK receptor antagonists, an interaction between the benzodiazepine with CCK-antagonist L-365, 260 [F(3, 56)= 4.2, p<0.05], but not with caerulein [F(1, 28)= 0.44, p<0.5] or with the CCK antagonist MK=329 [F(1, 28)= 1, 62, p>0.05] was observed. Further analysis shows that only the dose of 0.05 mg/kg of L-365, 260 reduced tolerance to diazepam response (Table II).

Effect of different doses of L-365, 260 on expression of tolerance to diazepam

Table III indicates the effect of different doses of CCK-B receptor antagonist L-365, 260 on diazepam-induced tolerance. When animals were treated with a dose of L-365, 260 (0.05, 0.25, 0.5 or 1 mg/kg, IP) 40 min. before administration of the test dose of diazepam, there was no interaction between the diazepam response in the presence or absence of L-365, 260 [two-way ANOVA; F(4, 54)= 0.44, p>0.05].

However, one-way ANOVA indicates that there is a difference between the response of animals treated with L-365, 260 in the presence or absence of diazepam. Analysis showed that L-365, 260 causes a delay in induction of seizure [F(8, 81)= 8.11, p<0.0001] and increases the seizure score [F(8, 81)= 15.2, p<0.0001].

DISCUSSION

In the present study, chronic administration of different doses of diazepam–induced tolerance to the anti-pentylenetetrazol (anti-PTZ) effect of the drug in mice. The results are in agreement with that found by others[21] which have shown that tolerance can be obtained to benzodiazepines.

It is established that chronic benzodiazepine administration can produce tolerance.[6,19] Tolerance does not develop uniformly, but rather depends on (1) the measure of drug action under study, (2) the particular drug used for chronic treatment[26,33] (3) the regional variation in CNS response to chronic treatment[15,18,25] and (4) the particular drug used for testing. Tolerance to the anti-pentylenetetrazol (anti-PTZ) effect of diazepam was shown by its decreased

Table III: Effect of different doses of L-365 260 on expression of tolerance induced to diazepam.

Drug Treatment	Induction of Ataxia	Ataxia Score	Induction of Seizure	Seizure Score
Vehicle 5 mL/kg	83.3±5.5	1.5±0.2	103.3±3.3	2.0±0.3
L-365.260 (0.05)	62.5±3.6	2.0±0.0	0.0±0.0	0.0±0.0
L-365.260 (0.25)	51.7±3.1	2.0±0.0	0.0±0.0	0.0±0.0
L-365.260 (0.5)	54.2±10.3	2.0±0.0	0.0±0.0	0.0±0.0
L-365.260 (1.0)	66.6±6.1	1.8±0.1	0.0±0.0	0.0±0.0
L-365.260 (0.05)+Diazepam	103.3±7.6*	1.5±0.2	220.2±20	2.3±0.3**
L-365.260 (0.25)+Diazepam	103.3±7.6**	1.5±0.4	220±19.0	2.3±0.2**
L-365.260 (0.5)+Diazepam	85.9±9.2	1.8±0.2	570±118.9**	1.7±0.5
L-365.260 (1.0)±Diazepam	115±5.0**	1.8±0.2	390±177.0*	2.0±0.5**

All mice were injected diazepam as described in Table I in order to induce tolerance. The animals were given (IP) vehicle, L-365.260 alone or L-365.260 40 min before diazepam on day 4 (test day). On the test day each animal received a test dose of diazepam (5 mg/kg) for ataxia evaluation of diazepam (5 mg/kg) 10 min before pentylenetetrazol (100 mg/kg, IP) administration for evaluation of the anticonvulsant effect of diazepam. Each point is the mean±SEM of 10 animals. *p<0.01, **p<0.001, different from respective saline control group.

ability to suppress PTZ seizures in rats that had been treated for a week with flurazepam.[21]

It has been shown that up-regulation of sulphated [^{3}H] CCK-8 binding can be found after chronic diazepam administration.[7] It has been proposed that chronic benzodiazepine administration induces changes in brain CCK neurotransmission.[1-7]

The presence of cholecystokinin octapeptide (CCK-8) in peripheral tissues and the central nervous system (CNS) has been demonstrated by several investigators.[2,29,14] The regional distribution of CCK-like neurons and CCK-8 sulfate concentrations in the brain have been described with immunocytochemical techniques and radioimmunoassay, respectively. CCK concentrations are particularly high in the cerebral cortices and limbic structures, brain areas which have been associated with cognitive processes, emotion and motivation.[31]

CCK functions at least through two distinct types of binding sites. These have been referred to as type A and type B receptors.[16]

In the present work, the effects of CCK receptor agonist and antagonists on diazepam-induced tolerance were examined. The present data indicated that the CCK receptor agonist caerulein[10,30,8] does not influence diazepam-induced tolerance.

The present data showed that the CCK-A receptor antagonist MK-329[3,9,17,32,32] did not alter tolerance induced by diazepam. However, CCK-B receptor antagonist (CT-988) is consistent with its ability to antagonize benzodiazepine receptor-mediated anxiogenic-like behaviour in animals.[23,24] This may support our results, however, the exact mechanism by which CI-988 antagonizes the diazepam-induced withdrawal proconvulsant effect is unclear.

To clarify the exact influences of CCK receptor mechanism(s) on diazepam tolerance, more experiments are required.

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