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Use of the specific benzodiazepine antagonist, Ro 15-1788, in studies of physiological dependence on benzodiazepines

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Summary. The specific benzodiazepine antagonist, Ro 15-1788, elicited withdrawal symptoms in squirrel monkeys, cats, rats and mice made tolerant and physically dependent by subchronic administration of high doses of diazepam, lorazepam or triazolam.

Physiological (physical) drug dependence is the altered physiological state which results from the long-lasting presence of high drug concentrations in the body and which necessitates the maintained administration of the drug in order to prevent the appearance of unpleasant somatic and/or psychic symptoms[1]. For most drugs, the only practicable way of detecting and quantifying physiological dependence in animals is to search for, and to measure, withdrawal (abstinence) symptoms that appear upon abrupt cessation of repeated drug administration. The occurrence, intensity and profile of the abstinence syndrome are, however, not a simple function of the severity of the state of physiological dependence, but also depend critically on the rate at which the pharmacological effects of the last drug dose disappear; this rate is in turn determined by the kinetics of dissociation of the drug from its receptors, the reversibility of the cellular events that are induced by the drug-receptor interaction, and the rate of elimination and/or metabolic inactivation of the drug. It is evident that the intensity of withdrawal symptoms should be most pronounced when the drug, which induces physiological dependence, is displaced from its receptors within the shortest possible time. Until recently opiates were the only psychotropic drugs for which specific antagonists were available. The dramatic withdrawal symptoms that are precipitated in opiate-dependent animals by opiate antagonists, such as naloxone, greatly contributed to the view of opiates being the drugs with a dependence liability par excellence.

Many other centrally (and even peripherally) acting agents are also capable of inducing physiological dependence under certain conditions. Among them, barbiturates lead to a type of physiological dependence which is clearly different from that induced by opiates. A physiological dependence similar in quality to, but milder in intensity than, the barbiturate type is induced by e.g. meprobamate and benzodiazepines[2]. Recently we have found potent and highly specific blockers of benzodiazepine receptors[3-6]; we anticipated that such compounds should be able to precipitate withdrawal symptoms in animals made physiologically dependent on benzodiazepines. We here report our findings with the specific benzodiazepine antagonist, Ro 15-1788.

Methods. Mice (SPF albino Füllinsdorf, 18–20 g), rats (SPF albino Füllinsdorf, 70–90 g) and mongrel cats (2.5–4.8 kg) of either sex as well as male squirrel monkeys (0.8–1.3 kg) were used. Mice and rats were administered a daily dose of 10 or 100 mg/kg diazepam p.o. for 12 days. Cats and monkeys were given a fixed dose of diazepam, lorazepam, triazolam or midazolam i.p. or p.o. for 15, 16, 18, or 21 days. The benzodiazepine antagonist, Ro 15-1788, dissolved in 0.3% v/v Tween 80 in distilled water, was injected i.v. or i.p. at various times after the last dose of the benzodiazepines. The animals were observed until withdrawal symptoms disappeared; the monkeys were controlled for 72 h by video-recording. In 3 cats, the bipolar EEG of the parietal and occipital cortex and dorsal hippocampus was also recorded.

Results. All doses of the benzodiazepines used were considerably higher than effective anxiolytic and anticonvulsant doses; they produced acute effects such as ataxia or decrease of vigilance. Complete or nearly complete tolerance to the latter effects developed in all species with repeated administration (diazepam and lorazepam within 5–8 days, triazolam within 3–5 days and midazolam within 3 days.

Withdrawal symptoms induced by 10 mg/kg Ro 15-1788 i.v. in mice and rats treated for 12 days with diazepam (10 or 100 mg/kg p.o. daily) are listed in the table. In mice, seizures lasting 2–3 sec occurred about 15 min after injection of the antagonist. All other symptoms appeared a few minutes after injection of the antagonist and lasted approximately 30 min. In rats, exophthalmos and a decrease of respiratory rate were seen for 45 min and 1.5–2 h, respectively.

In cats (N = 2) injected with 10 mg/kg lorazepam i.p. twice daily or 1 mg/kg triazolam i.p. once daily for 16 days, 100 mg/kg Ro 15-1788 i.p. either immediately or 1.5 h, 6 h, 12 h, 48 h or 60 h after the last dose evoked rigidity, vocalization and tachypnoe for 30 min as well as hypersalivation for 2 h (clonic seizures were observed only in the triazolam group, when Ro 15-1788 was injected 1.5 h after the last dose). Ro 15-1788 elicited similar withdrawal symptoms in cats treated with 30 mg/kg diazepam i.p., but only when injected within 12 h after the last dose; seizures were not observed. However, in 3 cats spike-wave activities

Changes in free behavior induced by 10 mg/kg Ro 15-1788 i.v. in mice and rats treated for 12 days with diazepam

Species and dose (p.o.)	Time of injection of Ro 15-1788[a]	Symptoms[b] Hypermotility	Increased muscle tone (rigidity)	Seizures Tonic	Clonic	Other symptoms[c]
Mouse, diazepam 10 mg/kg	0 h	4/12	3/12	0/12	1/12	TREMO 2/12, RERAD 6/12
	6 h	8/12	0/12	0/12	1/12	PILOE 1/12
	24 h	12/12	0/12	1/12	5/12	PILOE 2/12
Mouse, diazepam 100 mg/kg	0 h	10/10	9/10	4/10	4/10	SALIV 4/10
	6 h	10/10	6/10	1/10	1/10	SALIV 3/10
	24 h	9/9	8/9	4/9	4/9	SALIV 6/9
Rat, diazepam 10 mg/kg	0 h	2/12	0/12	0/12	0/12	EXOPH 4/12, RERAD 4/12
	6 h	3/12	0/12	0/12	0/12	EXOPH 8/12, RERAD 4/12
	24 h	0/12	0/12	0/12	0/12	EXOPH 9/12, HYREA 8/12
Rat, diazepam 100 mg/kg	0 h	0/12	11/12	0/12	0/12	EXOPH 3/12
	6 h	1/12	1/12	0/12	0/12	RERAD 4/12
	24 h	0/12	0/12	0/12	0/12	EXOPH 8/12, RERAD 5/12

[a]Time elapsed between 12th dose of diazepam and the i.v. injection of Ro 15-1788; [b]No. of animals showing the symptoms/number of animals per group; [c]TREMO, tremor; SALIV, hypersalivation; RERAD, respiratory rate decrease; EXOPH, exophthalmos; PILOE, piloerection; HYREA, hyperreactivity.

appeared for at least 2 h in the EEG of the parietal and occipital cortex but not of the dorsal hippocampus, when Ro 15-1788 was injected 2 h after the 18th daily dose of diazepam.

Two monkeys receiving 10 mg/kg diazepam p.o. for 15 days and injected with 10 mg/kg Ro 15-1788 i.v. either 1 h, 3 h, 12 h, 24 h or 48 h after the last dose showed vocalization for 3 min and thereafter increased motility, hypersalivation, loss of reactivity to the environment and short episodes of tremor as well as clonic convulsions for about 1 h. In addition, refusal of food was observed up to 4 h after injection of the antagonist. Monkeys treated once (N = 2) or twice daily (N = 2) with 30 mg/kg lorazepam p.o. exhibited similar withdrawal symptoms, when Ro 15-1788 was injected 1 h after the last dose. One of the monkeys, treated once daily, showed a clonic seizure 50 h after the last dose of lorazepam. Rigidity, loss of reactivity and refusal of food were seen in 2 monkeys for 1 h, when Ro 15-1788 was given 48 h after a 15 days treatment with 3 mg/kg triazolam p.o. No withdrawal symptoms were precipitated by Ro 15-1788 under the same test conditions in 2 monkeys treated with 30 mg/kg midazolam p.o.

Discussion. The specific benzodiazepine antagonist, Ro 15-1788, very rapidly and completely reverses all the acute pharmacological effects of various benzodiazepines in animals[3-6]. The present results indicate that this benzodiazepine receptor blocking property of Ro 15-1788 can be used, as expected, to precipitate withdrawal signs in animals made physiologically dependent on various benzodiazepines. Due to the rather long elimination half-life of most of these drugs, withdrawal symptoms upon cessation of chronic drug overdosing are usually mild, develop slowly and with variable latencies[1]. Ro 15-1788, by displacing within a few minutes the active benzodiazepines from their receptors, has the property of precipitating the abstinence symptoms at the time predetermined by the experimentor and of greatly amplifying their intensity. The disadvantage, of course, is that the compound creates a highly artificial situation, which leads to an overestimate of the severity of benzodiazepine dependence relative to the clinical situation and to the dependence liability of those drugs for which no specific antagonists are available.

The present results show that the profile and the intensity of precipitated withdrawal symptoms in animals made dependent on benzodiazepines differ in the 4 species studied; the symptoms were surprisingly mild in rats and consisted mainly of parasympathetic hyperactivity in cats. Transient seizures occurred in mice and squirrel monkeys. The duration of the withdrawal symptomatology was rather short; in part this may be accounted for by the relatively short duration of action of Ro 15-1788, although this point requires further experiments with repeated administration of the antagonist. Withdrawal precipitation occurred after diazepam, lorazepam and triazolam, but was absent in animals pretreated with the short acting midazolam[7]. It seems reasonable to predict that physiological dependence can be induced in principle by all available benzodiazepines, provided the dosage regimen takes into account the relative pharmacological potencies and the pharmacokinetic properties of the individual representatives; with very short acting benzodiazepines, such as midazolam, more frequent administrations than used here may be necessary, however, once dependence has been achieved, spontaneous abstinence symptoms may be more impressive than with longer acting derivatives. Although only 2 doses of diazepam were investigated so far in mice and rats, both of which were higher than required for obtaining pharmacological effects relevant to therapy, it can be concluded that the intensity of precipitated withdrawal symptoms clearly depends on the dose. The part played by other experimental variables in the precipitated withdrawal requires more extensive studies, namely the dose of Ro 15-1788 and the interval between the last benzodiazepine dose and the administration of the antagonist. Specific benzodiazepine antagonists appear to be powerful tools for studying the mechanisms involved in the development of physiological dependence.

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