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Indirect dopaminergic effects of tofisopam, a 2,3-benzodiazepine, and their inhibition by lithium

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Tofisopam, a 2,3-benzodiazepine, has been shown to have anxiolytic activity. However, in contrast to the widely used 1,4-benzodiazepines, it has no anticonvulsant, sedative or muscle relaxant effects. Tofisopam enhanced the behavioural actions of various dopaminergic drugs, both direct agonists, such as apomorphine (climbing behaviour in mice), and indirect agonists, such as (+)-amphetamine and amineptine (jumping behaviour in mice). Chronic treatment with lithium abolished the tofisopam-induced increase in the activity of these dopaminergic drugs. Thus tofisopam appears to induce acutely an increase in the sensitivity of central dopaminergic receptors which can be prevented by pretreatment with lithium.

The therapeutic potency of various 1,4-benzodiazepines (e.g. diazepam) correlates positively with their ability to displace radiolabelled benzodiazepines from their binding sites in the central nervous system (Möhler & Okada 1978). Tofisopam, a 2,3-benzodiazepine, which has been shown to have anxiolytic activity (Varaday et al 1975; Goldberg & Finnerty 1979; Pakkanen et al 1980) does not, however, displace the binding of labelled benzodiazepine but increases [^{3}H]flunitrazepam binding in-vitro and in-vivo (Saano et al 1981). In addition, and in contrast to the widely used 1,4-benzodiazepines, tofisopam has no anticonvulsant, sedative or muscle relaxant effects (Petöcz & Kosoczky 1975; Varady et al 1975; Seppalä et al 1980). Indeed, tofisopam may have a mild stimulant action (Gerevich et al 1975; Varady et al 1975; Bond & Lader 1982). To investigate further the potential stimulant action of tofisopam we have tested the behavioural effects of tofisopam mediated through brain dopamine receptors in control and in lithium-pretreated animals.

Materials and methods

Animals. Male, SW mice (St-Denis de Pile, France) in groups of 10 were housed under standard laboratory conditions at 20 ± 1 °C and 50% humidity with free access to food and water.

Mouse jumping. The experiment was carried out as described by Lal et al (1976). Mice, 18–20 g, were given tofisopam (50 mg kg^{-1} p.o.). Thirty min later each mouse was injected with 4 mg kg^{-1} i.p. (+)-amphetamine or 50 mg kg^{-1} i.p. amineptine, followed 15 min later by an injection of L-dopa (400 mg kg^{-1} i.p.) suspended in carboxymethylcellulose (CMC) 2%. Sixty min after the first drug administration (tofisopam) the number of jumps was counted during a 10 min observation period.

Climbing behaviour in mice. Four hours after an administration of tofisopam (50 mg kg^{-1} p.o.) or vehicle, mice, 18–20 g, were injected with 3 mg kg^{-1} s.c. apomorphine and were put into wire grid cages in pairs. Fifteen min and 30 min later the animals were observed for 30 s. The climbing behaviour was scored according to Protais et al (1976). The scores of two observations were combined.

Lithium treatment. Mice treated with lithium received 0·5 g litre^{-1} of lithium carbonate in the drinking water. The treatment period was 15 days. The daily quantity of lithium consumed by the mice was 3·36 ± 0·09 mg.

Drugs. The drugs used were: (+)-amphetamine hydrochloride (De Laire Chimie SA Calais, France), amineptine (Survector, Eutherapie, France), apomorphine hydrochloride (Sandoz, Basle, Switzerland), tofisopam (P.F. Medicaments, Castres, France). Tofisopam and amineptine were suspended in a 5% aqueous solution of polysorbate (Tween) 80. Apomorphine was dissolved in distilled water containing 0·1% ascorbic acid. The drugs were administered i.p. or s.c. at 10 ml kg^{-1} or through a stomach tube (p.o.) at 25 ml kg^{-1}.

Results

Jumping behaviour in mice (Table 1). An injection of L-dopa (400 mg kg^{-1} i.p.) in amphetamine-(4 mg kg^{-1} i.p.) or amineptine-(50 mg kg^{-1} i.p.) pretreated animals resulted in jumping behaviour. An injection of L-dopa in tofisopam-(50 mg kg^{-1} p.o.) pretreated mice did not induce the jumping behaviour. The number of jumps elicited by an injection of L-dopa in amphetamine- or amineptine-pretreated mice was, however, significantly increased by 50 mg kg^{-1} p.o. of tofisopam. The lithium treatment abolished the tofisopam-induced increase in amphetamine- or amineptine-pretreated mice.

Climbing behaviour in mice (Table 2). Tofisopam alone did not induce any climbing behaviour. The climbing behaviour induced by apomorphine (3 mg kg^{-1} s.c.) was, however, significantly increased by tofisopam (50 mg kg^{-1} p.o.). This effect was abolished by the lithium pretreatment.

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Table 1. The effects of tofisopam on jumping behaviour in mice. One hour after the injection of tofisopam or vehicle, the number of jumps was counted for a 10 min observation period. Amphetamine was given to 15 mice and amineptine to 6 mice. For lithium pretreated groups n = 15. The lithium treatment was for 15 days.

Drugs (mg kg^{-1})	Number of jumps	%
Water-pretreated mice		
(+)-Amphetamine 4 i.p. and L-dopa 400 i.p. + H_2O p.o. (1)	65·1 ± 23·2	—
or tofisopam 50 p.o.	259·7 ± 66·4*	+298·7
Amineptine 50 i.p. and L-dopa 400 i.p. + H_2O p.o. (3)	108·8 ± 26·6	—
or tofisopam 50 p.o.	330·5 ± 22·7**	+203·7
Lithium-pretreated mice		
(+)-Amphetamine 4 i.p. and L-dopa 400 i.p. + H_2O p.o. (2)	110·1 ± 39·3	—
or tofisopam 50 p.o.	115·1 ± 31·3	+4·8
Amineptine 50 i.p. and L-dopa 400 i.p. + H_2O p.o. (4)	107·5 ± 34·0	—
or tofisopam 50 p.o.	91·1 ± 28·5	−15·3

(2) ⟷ (1): NS, (3) ⟷ (4): NS.
*$P < 0·01$, **$P < 0·001$ when compared using Student's t-test with animals not receiving tofisopam.

Table 2. The effects of tofisopam on climbing behaviour induced by apomorphine in mice. Four hours after administration of tofisopam or vehicle (p.o.), mice were injected s.c. with 3 mg kg^{-1} apomorpine. The animals were observed for 30 s, 15 min and 30 min later. The lithium treatment was for 15 days. For the water pretreated mice n = 12 and for the lithium pretreated mice n = 20.

Drugs (mg kg^{-1})	Climbing score	%
Water-pretreated mice		
Apomorphine 3 s.c. + H_2O p.o. (1)	2·1 ± 0·4	—
Apomorphine 3 s.c. + tofisopam 50 p.o.	3·8 ± 0·7*	+84·0
Lithium-pretreated mice		
Apomorphine 3 s.c. + H_2O p.o. (2)	2·4 ± 0·4	—
Apomorphine 3 s.c. + tofisopam 50 p.o.	1·9 ± 0·4	−22·9

(1) ⟷ (2) NS.
*$P < 0·05$ compared using Student's t-test with the animals not receiving tofisopam.

Discussion

Tofisopam produced an enhancement of the action of various dopaminergic drugs, both direct agonists, such as apomorphine (climbing behaviour in mice) and indirect agonists, such as (+)-amphetamine and amineptine (jumping test in mice). These results suggest that tofisopam might act by increasing the sensitivity of central dopamine receptors. The climbing behaviour is considered to reflect stimulation of striatal dopamine receptors (Protais et al 1976; Costall et al 1978) and might be used to quantify the effects of apomorphine (Costentin et al 1975; Von Voigtlander et al 1975). Moreover, dopamine receptors which are involved in the climbing response can be hypersensitized but not desensitized (Cools & van Rossum 1980). On the other hand, Lal et al (1976) have attributed amphetamine- and L-dopa-induced mouse jumping to dopaminergic overstimulation and have cited the inhibition of jumping by neuroleptics as evidence for the specific mediation of jumping behaviour by dopamine systems.

The apparent potentiation of dopaminergic responses may be mediated through a modification of postsynaptic dopamine receptors or alternatively by sensitization of neurons downstream from striatal dopamine receptors. The results with lithium suggest that a potentiation of the level of the dopamine receptors may be more likely.

Chronic treatment with lithium abolished the tofisopam-induced increase in the activity of the dopaminergic drugs studied here. Lithium treatment has been shown to produce a significant decrease in the number of dopamine receptors in rat striatum (Rosenblatt et al 1980; Verimer et al 1980) and prevents the increase in dopamine receptor sensitivity induced by chronic haloperidol treatment (Klawans et al 1977; Pert et al 1978). Lithium prevention of receptor sensitivity changes appears to be unidirectional since lithium can prevent receptor supersensitivity, but not receptor subsensitivity (Birmaher et al 1982). Thus the inhibition of the tofisopam-induced potentiation of dopaminergic activity by lithium could result from a prevention of the receptor-supersensitivity normally induced by tofisopam. In-vitro, tofisopam does not change the specific binding of [^{3}H]spiroperidol to dopamine receptors in the rat brain (M. Charveron, unpublished results).

An indirect stimulation by tofisopam has been shown in other neurotransmitter systems. Tofisopam has no affinity for benzodiazepine receptors but increases their affinity for 1,4-benzodiazepines such as diazepam (Saano & Airaksinen 1982; Saano & Urtti 1982). The responses to diazepam against tremor and convulsions are selectively potentiated by tofisopam (Saano et al 1983; Briley et al 1984). In addition, tofisopam has been shown to modulate GABA receptor binding, enhancing GABA receptor binding to the same extent as 1,4-benzodiazepines (Saano 1982). Other studies have suggested that tofisopam increases the maximum number of benzodiazepine binding sites without affecting ligand affinity (Mennini et al 1982).

Thus, in summary, tofisopam appears to induce acutely a dopamine receptor supersensitivity which can be prevented by prior treatment with lithium. The stimulatory activity of tofisopam on the dopaminergic system described here is consistent with clinical reports (Gerevich et al 1975; Bond & Lader 1982) of a mild stimulant effect in contrast to the greater or lesser sedative effects seen with 1,4-benzodiazepines.

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Extraction efficiency and biliary excretion of hepatobiliary imaging agents in the rat perfused liver

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A multitude of transitional metal complexes has recently been introduced into clinical practice as hepatobiliary imaging agents; the kinetics of these substances are often poorly understood. To gain better insight into characteristics of these different agents, we measured the extraction efficiency and mean biliary transit time of a variety of iminodiacetate derivatives in the in-situ rat perfused liver. First pass hepatic extraction efficiency averaged 59% for ^{99m}Tc *N*-(*p*-isopropylacetanilide) iminodiacetate, 73% for ^{99m}Tc *N*-(2,6-diethylacetanilide) iminodiacetate, 74% for ^{99m}Tc *N*-(3-bromo-2,4,6-trimethylacetanilide) iminodiacetate, 90% for ^{99m}Tc *N*-(*p*-butylacetanilide) iminodiacetate, and 93% for ^{99m}Tc *N*-pyridoxyl-5-methyltryptophan. By comparison, extraction of another organic anionic compound, ^{131}I Rose bengal, was only 12·4%. Mean hepatocyte transit times varied from 2·3 to 7·5 min. Shorter mean transit times were observed for di*ortho* substituted and longer mean transit times for *para* substituted metal complexes. Radioactivity was quantitatively recovered in bile, and excretion kinetics overall were consistent with data generated in whole animals. These studies demonstrate the value of the in-situ rat perfused liver as a screening tool to characterize hepatobiliary imaging agents.

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The ideal hepatobiliary imaging agent should meet the following criteria: (Chervu et al 1982) efficient hepatic uptake (Fritzberg & Klingensmith 1982), short biliary transit time (Wistow et al 1977), specificity for hepatobiliary excretion in patients with both normal and decreased hepatocellular function and (Fritzberg et al 1982) adequate properties as far as imaging is concerned (Chervu et al 1982; Fritzberg & Klingensmith 1982). In the early 70s, a class of chelating agents which met most of these criteria was described (Chervu et al 1982). These agents were found to form complexes with the transition metal, technetium, whose 99m isotope provides high quality gamma camera images with a low radiation dose to the patient because of its abundance of 140 keV photons, low particulate emission and short half life of 6 h. Characterization of these different ^{99m}Tc complexes have involved collection of blood, bile and urine in baboons (Wistow et al 1977), rats (Fritzberg et al 1982) or recording time activity curves over regions of interest in rabbits (Nunn et al 1981). These methods, however, usually do not provide a direct measurement of hepatic extraction efficiency