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Influence of Certain Calcium-channel Blockers on Some Aspects of Lorazepam-dependence in Mice

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Abstract

The effect of acute and chronic treatments of the calcium-channel blockers, isradipine, diltiazem and flunarizine in protecting against lorazepam dependence has been demonstrated in mice.

Dependence was induced by twice-daily administration of lorazepam (1 mg kg^{-1}) for 10 days, doubling the dose during the next 10 days. Withdrawal symptoms and changes in the noradrenaline, dopamine and 5-hydroxytryptamine content of different regions of the brain were observed after either 24-h withdrawal or flumazenil administration. Isradipine inhibited lorazepam withdrawal symptoms, the effect being accompanied in the 24-h withdrawal group by significant decreases in the noradrenaline and dopamine content of the thalamus and hypothalamus and in the noradrenaline content of the mid-brain. In the flumazenil-treated group isradipine produced significant decreases in mid-brain noradrenaline and dopamine levels and in the dopamine content of the thalamus and hypothalamus. Diltiazem did not, on the other hand, afford significant protection against lorazepam withdrawal symptoms and did not induce any significant change in the neurotransmitters studied. Flunarizine significantly inhibited lorazepam withdrawal symptoms, an effect accompanied by significant reduction in noradrenaline and dopamine levels in the thalamus and hypothalamus. Dopamine was also significantly reduced in the cerebral cortex. Similar effects were produced in the flumazenil-treated group, and the noradrenaline content was reduced in the medulla, pons and cerebellum.

It was concluded that isradipine and flunarizine might be of value in ameliorating lorazepam withdrawal symptoms.

Benzodiazepines were introduced in clinical practice in 1960. They have been widely prescribed and were originally considered safe drugs. Their massive use eventually led to awareness that severe withdrawal states could develop after termination of high multiple dosage (Clare 1971). Systematic studies revealed induction of drug-dependence (Petursson & Lader 1986). In 1988, firm and authoritative warnings of benzodiazepine dependence were issued (Committee on Safety of Medicines 1988). Although dependence was directly related to the dose and duration of treatment (Lukas & Griffiths 1984), benzodiazepines have low abuse potential when compared with other abused drugs (Woods et al 1987). The development of specific benzodiazepine-receptor antagonists (Hunkeler et al 1981) has facilitated the study of this dependence (Cumin et al 1982).

Benzodiazepine-dependence is probably accompanied by changes in brain neurotransmitters. It was reported that high doses of benzodiazepines reduce noradrenaline turnover (Taylor & Laverty 1969) and a correlation was suggested between their dependence and brain noradrenaline. It was reported that diazepam, bromazepam, oxazepam, lorazepam and chlordiazepoxide withdrawal symptoms were accompanied by a significant increase in the noradrenaline level in the cerebral cortex and in the thalamus and hypothalamus of mice (Saad & Attia 1991). Although dopamine content was not affected by benzodiazepine treatment, there is indirect evidence of an effect on its metabolism (Ida & Roth 1987).

An increase in 5-hydroxytryptamine (5-HT) functions in the brain has been reported to produce symptoms similar to those seen during benzodiazepine withdrawal (Zohar et al 1988). γ-Aminobutyric acid probably also plays an important role in benzodiazepine-dependence because turnover studies revealed a significant reduction in its synthesis during withdrawal (Saad et al 1995).

Medical treatment of drug dependence is of major importance. It has been reported that certain members of the dihydropyridine calcium-channel blockers inhibit the withdrawal symptoms of some abused drugs. Thus nitrendipine was effective in treating barbiturate (Brown et al 1988) and flurazepam (Dolin et al 1990) dependence. Similarly, nimodipine, dantrolene and nitrendipine were effective in suppressing ethanol withdrawal tremors (Bone et al 1989; Littleon et al 1990).

The aim of this work was to investigate experimentally the potential usefulness of certain calcium-channel blockers in protecting against lorazepam dependence in mice, because calcium plays an important role in drug dependence (Yamamoto et al 1978) and its influx results in release of several neurotransmitters including noradrenaline from nerve terminals. The drugs used were isradipine, diltiazem and flunarizine.

Materials and Methods

Animals

Adult male albino mice, 20–25 g, were obtained from El Nasr company, Cairo, Egypt. The animals were housed in colony cages (ten mice/cage) under constant environmental conditions with free access to food (standard pellets) and water.

Drugs and chemicals

Lorazepam was obtained from Wyeth–Pharma, Munster, Germany; the doses used were 1 and 2 mg kg^{-1}. Flumazenil

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was obtained from Hoffmann la Roche, Basel, Switzerland; the dose used was 4 mg kg^{-1}. Flunarizine HCl was obtained from Janssen Pharmaceutical, Belgium; the doses used were 1 and 2 mg kg^{-1}. Diltiazem HCl was obtained from Knoll, Heidelberg, Germany; the doses used were 10 and 20 mg kg^{-1}. Isradipine was obtained from Sandoz, Basle, Switzerland; the doses used were 0·6 and 1·2 mg kg^{-1}.

All drugs were dissolved either in saline or with a small amount of Tween 80 and were administered intraperitoneally. Noradrenaline was obtained from Serva (Heidelberg, Germany), dopamine from Sigma (St Louis, USA) and 5-HT from BDH (Poole, UK). All other chemicals were of analytical-reagent grade.

Experimental design

Mice were rendered dependent by twice daily intraperitoneal administration of lorazepam at 1 mg kg^{-1} for 10 days, doubling the dose during the next 10 days (Galpern et al 1990; Saad et al 1995). Withdrawal symptoms were recorded in different groups of mice (n = 12), either 24 h after lorazepam withdrawal, or by the intraperitoneal administration of flumazenil (4 mg kg^{-1}), 2 h after the last injection of lorazepam. Behavioural changes (hypermotility, rigidity and convulsions) were recorded for 15 min according to the method described by Cumin et al (1982). A score or no score was given for each mouse and the scores of each group were added and the results analysed statistically.

Effect of calcium-channel blockers on 24 h lorazepam-induced withdrawal symptoms

Acute treatment. Isradipine (0·6 and 1·2 mg kg^{-1}), diltiazem (10 and 20 mg kg^{-1}) or flunarizine (1 and 2 mg kg^{-1}) were administered intraperitoneally to groups of lorazepam-dependent mice (n = 6), 22 h after withdrawal of lorazepam. Behavioural tests and determination of noradrenaline, dopamine and 5-HT content were conducted 2 h later.

Chronic treatment. The calcium-channel blockers were administered intraperitoneally, daily, during the last 6 days of lorazepam-induced dependence. The seventh dose of the calcium-channel blocker was given 22 h after withdrawal of lorazepam. Decapitation for determination of brain monoamines was performed 2 h after the last dose of the calcium-channel blocker, and 24 h after lorazepam withdrawal.

Effect of calcium-channel blockers on flumazenil-induced lorazepam withdrawal symptoms

Acute treatment. Each of the calcium-channel blockers was given 1 h after the last dose of lorazepam; flumazenil was then administered 1 h later. The mice were decapitated 1 h after administration of flumazenil.

Chronic treatment. Each of the calcium-channel blockers was administered intraperitoneally, daily during the last 7 days of lorazepam-induced dependence. Flumazenil was given 2 h after the last dose of lorazepam; mice were decapitated 1 h later. In all cases behavioural tests were conducted for each mouse during the last 15 min before decapitation.

Determination of the noradrenaline, dopamine and 5-HT content of mouse brain

Pooled samples, each from 2 mice killed by decapitation, were used for the determination of the noradrenaline, dopamine and 5-HT content of four different regions of the brain, the cerebral cortex, the thalamus and hypothalamus, the mid-brain and the medulla, pons and cerebellum, all of which were dissected from frozen brain in less than 1 min. A spectrophotofluorimetric method was used to determine these monoamines (Ciarlone 1978). Fluorescence was measured for noradrenaline, dopamine and 5-HT at the wavelengths 480, 375 and 470 nm, respectively, after excitation at 380, 320 and 355 nm, respectively.

Statistical analysis

The data were analysed by analysis of variance then by Dunnett's test and differences were considered significant if $P < 0.05$. Behavioural changes were analysed for significance using the χ^2 test, again $P < 0.05$ was taken to indicate significance.

Results

The effects of the different calcium-channel blockers on the monoamine content of different regions of the brain of 24-h withdrawal and of flumazenil-treated lorazepam-dependent mice are seen in Tables 1 and 2, respectively. The behavioural effects of withdrawal are listed in Table 3. Each drug was used in two doses, equivalent to therapeutic doses in man and transferred according to Paget & Barners (1964).

In the 24-h withdrawal group, isradipine significantly reduced the noradrenaline content of the thalamus and hypothalamus. In chronic experiments, it reduced the noradrenaline content of the thalamus and hypothalamus and of the mid-brain of both treated groups. No change was observed in noradrenaline levels in other brain regions. Chronic treatment with isradipine significantly reduced the dopamine content of the thalamus and hypothalamus in the 24-h withdrawal group. In the flumazenil-treated group dopamine content was reduced in the thalamus and hypothalamus and in the mid-brain. There was no significant change in the 5-HT content of the different brain regions. Treatment with isradipine significantly reduced withdrawal symptoms in both groups of lorazepam-dependent mice.

Diltiazem did not afford any protection against either the 24 h withdrawal symptoms or the flumazenil-induced symptoms in lorazepam-dependent mice and did not affect the noradrenaline, dopamine or 5-HT content of the different regions of the brain.

Flunarizine significantly reduced the noradrenaline content of the thalamus and hypothalamus region of the 24 h withdrawal group and the noradrenaline content of the thalamus and hypothalamus and medulla, pons and cerebellum of the flumazenil-treated group. Dopamine content was also reduced both in the cerebral cortex and in the thalamus and hypothalamus of both groups. No change in the 5-HT content of the

Table 1. Effect of isradipine, diltiazem and flunarizine on noradrenaline, dopamine and 5-HT content ($\mu g\ g^{-1}$ wet wt) in different regions of mouse brain 24 h after lorazepam withdrawal in dependent mice.

Treatment	Cerebral cortex			Thalamus and hypothalamus		
	Noradrenaline	Dopamine	5-HT	Noradrenaline	Dopamine	5-HT
Controls						
Saline	0·39 ± 0·05	0·56 ± 0·15	0·53 ± 0·15	1·83 ± 0·23	0·52 ± 0·11	0·9 ± 0·26
Tween 80	0·41 ± 0·06	0·66 ± 0·15	0·52 ± 0·15	1·65 ± 0·21	0·51 ± 0·11	0·87 ± 0·23
24-h withdrawal + isradipine	0·74 ± 0·06*	0·58 ± 0·17	0·50 ± 0·14	2·22 ± 0·19	0·64 ± 0·16	0·84 ± 0·22
Acute						
0·6 mg kg^{-1}	0·67 ± 0·09	0·70 ± 0·16	0·66 ± 0·17	1·82 ± 0·22†	0·64 ± 0·15	0·90 ± 0·23
1·2 mg kg^{-1}	0·63 ± 0·11	0·64 ± 0·15	0·50 ± 0·17	1·90 ± 0·20†	0·46 ± 0·16	0·78 ± 0·34
Chronic						
0·6 mg kg^{-1}	0·56 ± 0·08	0·61 ± 0·13	0·69 ± 0·21	1·49 ± 0·11†	0·51 ± 0·16	0·75 ± 0·15
1·2 mg kg^{-1}	0·44 ± 0·06	0·69 ± 0·14	0·72 ± 0·19	1·43 ± 0·08†	0·31 ± 0·05†	0·90 ± 0·18
24-h withdrawal + diltiazem						
Acute						
10 mg kg^{-1}	0·71 ± 0·14	0·68 ± 0·18	0·57 ± 0·17	2·3 ± 0·52	0·37 ± 0·17	0·78 ± 0·23
20 mg kg^{-1}	0·69 ± 0·16	0·70 ± 0·54	0·20 ± 0·22	1·9 ± 0·30	0·56 ± 0·17	0·99 ± 0·20
Chronic						
10 mg kg^{-1}	0·61 ± 0·12	0·66 ± 0·16	0·60 ± 0·17	2·14 ± 0·3	0·63 ± 0·16	0·63 ± 0·18
20 mg kg^{-1}	0·64 ± 0·16	0·68 ± 0·21	0·59 ± 0·15	2·3 ± 0·30	0·69 ± 0·18	0·80 ± 0·21
24-h withdrawal + flunarizine						
Acute						
1 mg kg^{-1}	0·76 ± 0·05	0·58 ± 0·16	0·45 ± 0·11	2·20 ± 0·29	0·54 ± 0·14	0·79 ± 0·19
2 mg kg^{-1}	0·73 ± 0·07	0·63 ± 0·14	0·47 ± 0·12	2·21 ± 0·43	0·60 ± 0·12	0·78 ± 0·14
Chronic						
1 mg kg^{-1}	0·50 ± 0·04	0·31 ± 0·06†	0·57 ± 0·13	1·41 ± 0·06†	0·38 ± 0·06†	0·84 ± 0·15
2 mg kg^{-1}	0·36 ± 0·04	0·26 ± 0·05†	0·45 ± 0·14	1·13 ± 0·08†	0·29 ± 0·06†	0·74 ± 0·14

Treatment	Mid-brain			Medulla, pons and cerebellum		
	Noradrenaline	Dopamine	5-HT	Noradrenaline	Dopamine	5-HT
Controls						
Saline	0·42 ± 0·05	0·39 ± 0·10	1·1 ± 0·35	0·19 ± 0·08	0·33 ± 0·02	0·83 ± 0·30
Tween 80	0·44 ± 0·05	0·45 ± 0·09	1·05 ± 0·28	0·20 ± 0·03	0·28 ± 0·07	0·96 ± 0·31
24-h withdrawal + isradipine	0·43 ± 0·08	0·44 ± 0·09	0·89 ± 0·30	0·24 ± 0·06	0·35 ± 0·09	0·69 ± 0·17
Acute						
0·6 mg kg^{-1}	0·37 ± 0·08	0·43 ± 0·11	0·95 ± 0·30	0·19 ± 0·03	0·38 ± 0·10	0·93 ± 0·27
1·2 mg kg^{-1}	0·32 ± 0·07	0·45 ± 0·11	1·03 ± 0·30	0·21 ± 0·04	0·47 ± 0·13	0·85 ± 0·30
Chronic						
0·6 mg kg^{-1}	0·28 ± 0·04†	0·53 ± 0·11	0·89 ± 0·19	0·28 ± 0·06	0·44 ± 0·09	0·69 ± 0·16
1·2 mg kg^{-1}	0·24 ± 0·03†	0·37 ± 0·07	1·07 ± 0·34	0·28 ± 0·06	0·39 ± 0·09	0·74 ± 0·16
24-h withdrawal + diltiazem						
Acute						
10 mg kg^{-1}	0·48 ± 0·07	0·37 ± 0·11	1·09 ± 0·35	0·27 ± 0·06	0·33 ± 0·10	0·82 ± 0·20
20 mg kg^{-1}	0·46 ± 0·11	0·57 ± 0·13	1·13 ± 0·39	0·28 ± 0·05	0·38 ± 0·09	1·02 ± 0·29
Chronic						
10 mg kg^{-1}	0·45 ± 0·11	0·43 ± 0·12	0·78 ± 0·20	0·26 ± 0·06	0·45 ± 0·11	0·58 ± 0·18
20 mg kg^{-1}	0·45 ± 0·12	0·38 ± 0·12	0·96 ± 0·22	0·3 ± 0·05	0·42 ± 0·11	0·85 ± 0·23
24-h withdrawal + flunarizine						
Acute						
1 mg kg^{-1}	0·41 ± 0·11	0·47 ± 0·11	0·78 ± 0·18	0·22 ± 0·04	0·31 ± 0·06	0·69 ± 0·18
2 mg kg^{-1}	0·39 ± 0·09	0·58 ± 0·15	0·82 ± 0·17	0·23 ± 0·05	0·28 ± 0·10	0·60 ± 0·16
Chronic						
1 mg kg^{-1}	0·40 ± 0·07	0·45 ± 0·09	0·81 ± 0·18	0·24 ± 0·05	0·46 ± 0·10	0·78 ± 0·20
2 mg kg^{-1}	0·45 ± 0·08	0·59 ± 0·12	0·94 ± 0·14	0·25 ± 0·05	0·47 ± 0·10	0·81 ± 0·14

Values are means ± s.e.m. from 6 pooled samples. *$P < 0·05$ compared with corresponding control. †$P < 0·05$ compared with the effect of 24-h withdrawal.

Table 2. Effect of isradipine, diltiazem and flunarizine on noradrenaline, dopamine and 5-HT content ($\mu g\ g^{-1}$ wet wt) in different regions of mouse brain 24 h after flumazenil treatment in dependent mice.

Treatment	Cerebral cortex			Thalamus and hypothalamus		
	Noradrenaline	Dopamine	5-HT	Noradrenaline	Dopamine	5-HT
Controls						
Tween 80	0·41 ± 0·05	0·66 ± 0·15	0·52 ± 0·15	1·65 ± 0·21	0·51 ± 0·11	0·87 ± 0·23
Saline	0·39 ± 0·05	0·56 ± 0·15	0·53 ± 0·15	1·83 ± 0·23	0·52 ± 0·11	0·9 ± 0·26
Flumazenil	0·55 ± 0·03	0·68 ± 0·20	0·58 ± 0·17	2·4 ± 0·20	0·49 ± 0·13	0·99 ± 0·25
Flumazenil + isradipine	1·36 ± 0·25*	0·68 ± 0·22	0·56 ± 0·16	3·10 ± 0·29*	0·70 ± 0·17	0·99 ± 0·31
Acute						
0·6 mg kg^{-1}	1·20 ± 0·23	0·73 ± 0·18	0·69 ± 0·19	3·16 ± 0·46	0·61 ± 0·17	0·83 ± 0·24
1·2 mg kg^{-1}	1·23 ± 0·23	0·53 ± 0·15	0·71 ± 0·16	2·69 ± 0·43	0·55 ± 0·13	0·92 ± 0·23
Chronic						
0·6 mg kg^{-1}	1·04 ± 0·23	0·57 ± 0·16	0·52 ± 0·17	2·43 ± 0·44	0·38 ± 0·08†	0·90 ± 0·22
1·2 mg kg^{-1}	0·83 ± 0·11	0·68 ± 0·11	0·63 ± 0·21	2·00 ± 0·81	0·35 ± 0·07†	0·85 ± 0·24
Flumazenil + diltiazem						
Acute						
10 mg kg^{-1}	1·49 ± 0·34	0·71 ± 0·17	0·63 ± 0·17	3·13 ± 0·39	0·74 ± 0·15	0·74 ± 0·19
20 mg kg^{-1}	1·59 ± 0·30	0·79 ± 0·19	0·54 ± 0·17	3·05 ± 0·35	0·73 ± 0·19	0·69 ± 0·17
Chronic						
10 mg kg^{-1}	1·57 ± 0·35	0·74 ± 0·20	0·50 ± 0·14	3·14 ± 0·46	0·78 ± 0·19	0·83 ± 0·29
20 mg kg^{-1}	1·42 ± 0·36	0·64 ± 0·22	0·59 ± 0·19	3·18 ± 0·46	0·67 ± 0·19	0·79 ± 0·22
Flumazenil + flunarizine						
Acute						
1 mg kg^{-1}	1·08 ± 0·32	0·62 ± 0·16	0·50 ± 0·16	2·80 ± 0·51†	0·55 ± 0·16	0·76 ± 0·13
2 mg kg^{-1}	1·12 ± 0·34	0·58 ± 0·14	0·62 ± 0·13	2·93 ± 0·54	0·56 ± 0·13	0·79 ± 0·16
Chronic						
1 mg kg^{-1}	1·53 ± 0·11	0·37 ± 0·06†	0·58 ± 0·14	1·60 ± 0·45†	0·37 ± 0·10†	0·74 ± 0·16
2 mg kg^{-1}	1·46 ± 0·10	0·35 ± 0·06†	0·63 ± 0·14	1·55 ± 0·16†	0·33 ± 0·06†	0·86 ± 0·16

Treatment	*Mid-brain*			*Medulla, pons and cerebellum*		
	Noradrenaline	Dopamine	5-HT	Noradrenaline	Dopamine	5-HT
Controls						
Tween 80	0·44 ± 0·05	0·45 ± 0·09	1·05 ± 0·28	0·20 ± 0·03	0·28 ± 0·07	0·96 ± 0·31
Saline	0·42 ± 0·05	0·39 ± 0·01	1·10 ± 0·35	0·19 ± 0·02	0·33 ± 0·08	0·83 ± 0·30
Flumazenil	0·40 ± 0·08	0·43 ± 0·06	1·02 ± 0·33	0·22 ± 0·03	0·31 ± 0·07	0·79 ± 0·26
Flumazenil + isradipine	0·53 ± 0·11	0·49 ± 0·12	1·08 ± 0·31	0·27 ± 0·06	0·41 ± 0·10	0·99 ± 0·31
Acute						
0·6 mg kg^{-1}	0·44 ± 0·06	0·65 ± 0·15	1·20 ± 0·31	0·99 ± 0·07	0·53 ± 0·11	1·09 ± 0·32
1·2 mg kg^{-1}	0·37 ± 0·08	0·52 ± 0·13	1·17 ± 0·25	0·34 ± 0·10	0·37 ± 0·10	1·20 ± 0·31
Chronic						
0·6 mg kg^{-1}	0·32 ± 0·06†	0·28 ± 0·06†	1·09 ± 0·32	0·30 ± 0·08	0·56 ± 0·10	1·19 ± 0·30
1·2 mg kg^{-1}	0·26 ± 0·05†	0·27 ± 0·05†	0·98 ± 0·19	0·28 ± 0·02	0·52 ± 0·11	1·28 ± 0·27
Flumazenil + diltiazem						
Acute						
10 mg kg^{-1}	0·58 ± 0·09	0·52 ± 0·13	0·83 ± 0·22	0·29 ± 0·07	0·35 ± 0·08	0·80 ± 0·21
20 mg kg^{-1}	0·47 ± 0·11	0·59 ± 0·17	0·90 ± 0·22	0·22 ± 0·06	0·51 ± 0·14	1·17 ± 0·63
Chronic						
10 mg kg^{-1}	0·48 ± 0·11	0·4 ± 0·11	0·99 ± 0·26	0·24 ± 0·06	0·41 ± 0·09	0·83 ± 0·17
20 mg kg^{-1}	0·46 ± 0·07	0·42 ± 0·12	1·18 ± 0·32	0·25 ± 0·06	0·47 ± 0·11	1·16 ± 0·35
Flumazenil + Flunarizine						
Acute						
1 mg kg^{-1}	0·38 ± 0·06	0·63 ± 0·13	1·07 ± 0·28	0·24 ± 0·05†	0·54 ± 0·12	0·94 ± 0·25
2 mg kg^{-1}	0·45 ± 0·08	0·53 ± 0·14	0·90 ± 0·22	0·27 ± 0·07	0·48 ± 0·17	0·89 ± 0·14
Chronic						
1 mg kg^{-1}	0·41 ± 0·08	0·46 ± 0·14	0·98 ± 0·15	0·26 ± 0·07	0·55 ± 0·15	0·73 ± 0·24
2 mg kg^{-1}	0·49 ± 0·09	0·61 ± 0·13	0·94 ± 0·15	0·24 ± 0·06†	0·53 ± 0·11	0·93 ± 0·19

Values are means ± s.e.m. from 6 pooled samples. *$P < 0.05$ compared with corresponding control. †$P < 0.05$ compared with the effect of flumazenil treatment.

Table 3. Percentage of total behavioural scores indicating withdrawal of lorazepam (24 h or flumazenil treatment) after acute and chronic treatment with isradipine, diltiazem and flunarizine.

			24-h withdrawal	Flumazenil treatment
Control			100	100
Isradipine	Acute	0.6 mg kg^{-1}	80	85
	Acute	1.2 mg kg^{-1}	65*	65*
	Chronic	0.6 mg kg^{-1}	85	70*
	Chronic	1·2 mg kg^{-1}	65*	55*
Diltiazem	Acute	10 mg kg^{-1}	120	110
	Acute	20 mg kg^{-1}	110	95
	Chronic	10 mg kg^{-1}	100	95
	Chronic	20 mg kg^{-1}	110	120
Flunarizine	Acute	1 mg kg^{-1}	120	90
	Acute	2 mg kg^{-1}	130	90
	Chronic	1 mg kg^{-1}	70*	50*
	Chronic	2 mg kg^{-1}	60*	45*

*$P < 0.05$ compared with the corresponding control (n = 12).

different regions of the brain was recorded. Behavioural changes indicated that chronic treatment with flunarizine afforded protection both against 24-h withdrawal symptoms and against flumazenil-induced symptoms in lorazepam-dependent mice.

Discussion

Previous studies have revealed that lorazepam is the most frequently abused drug among benzodiazepine-dependent persons (Wolf et al 1989). This drug has more potent withdrawal symptoms than benzodiazepines of longer duration (Saad & Attia 1991). The intensity of withdrawal is more pronounced when the drug is displaced from its receptors within the shortest possible time.

In this work two models were used to demonstrate lorazepam withdrawal, 24-h withdrawal, which mimics what normally occurs when a benzodiazepine-dependent patient stops drug administration, and administration of flumazenil, which has a high specific blocking effect on the benzodiazepine receptors and precipitates withdrawal symptoms in benzodiazepine-dependent man and experimental animals (Hunkeler et al 1981; Cumin et al 1982).

Comparing both groups, flumazenil-treated lorazepam-dependent mice showed a higher intensity of abstinence syndromes than the 24-h withdrawal group. This was accompanied by a greater increase in the noradrenaline content of the cerebral cortex (248·7% and 89·7%, respectively). The noradrenaline content of the thalamus and hypothalamus region of the flumazenil-treated group was increased by 69·4%, whereas no significant change was observed in the same region of the 24-h withdrawal group.

It is probable that there is an intimate correlation between the increase in noradrenaline content and the stress accompanying lorazepam withdrawal. Thus, it was previously reported that stress and electrical shock increase noradrenaline release in the cerebral cortex and hypothalamus, an action attenuated in a dose-dependent manner by diazepam and antagonized by flumazenil (Ida et al 1988). There is also evidence for a rebound noradrenergic activity in dependent animals (Rastogi et al 1978) or man (Nutt & Molyneux 1987) during withdrawal. Similarly, the results of Bickford–Wimer et al (1988) suggest increased noradrenergic activity during benzodiazepine withdrawal.

In this work three calcium-channel blockers of different chemical groups were used in mice. Isradipine is a dihydropyridine derivative. Widespread distribution of dihydropyridine-sensitive calcium channels in the central nervous system (CNS) has been demonstrated, with the highest density in the cerebral cortex and thalamus and hypothalamus regions (Gould et al 1985). Although it was reported by Miller & Friedman (1984) that these drugs have no marked effects on the CNS, their lipophilic character enables passage through the blood-brain barrier at micromolar levels, the concentration required to affect the CNS (Supervialai & Karobath 1984; Dolin et al 1986). Isradipine reduced lorazepam abstinence syndrome induced either by 24-h withdrawal or by flumazenil administration. This action was accompanied by reduced levels of noradrenaline and dopamine, but was without effect on 5-HT levels. It was reported that the affinity of dihydropyridine derivatives to 5-HT is very low (Adachi & Shoji 1986). This negative result should not, however, be taken as denying a possible role of 5-HT, and further turnover studies might be necessary. Several dihydropyridine calcium-channel blockers have been reported to induce an increase in brain 5-HT turnover in-vivo (Colado et al 1991).

Diltiazem, a thiobenzadiazepine derivative, was found not to affect any of the tested monoaminergic neurotransmitters in different regions of the brain during lorazepam-induced abstinence syndrome. Neither did it afford any significant protection against lorazepam withdrawal symptoms. Similarly, Mizoguchi et al (1993) reported the inability of diltiazem to affect diazepam withdrawal symptoms. Literature reports on the central effects of diltiazem are very few. It has been reported that diltiazem does not affect either the striatal dopaminergic system (Ikegami et al 1992) or the 5-HT-ergic system (Colado et al 1993).

Flunarizine, a piperazine derivative, was reported to be a calcium-channel antagonist in several tissues including brain (Binnie 1989), with predominant specificity at the L-type channel (Iwamoto et al 1991) and to a lesser extent at the T-type channel (Mizoguchi et al 1993).

Chronic treatment with flunarizine significantly reduced the noradrenaline and dopamine content of the thalamus and hypothalamus, and the dopamine content of the cerebral cortex, after 24 h withdrawal from lorazepam. Similarly, flunarizine caused a significant reduction in the noradrenaline content of the thalamus and hypothalamus and of the medulla, pons and cerebellum in flumazenil-treated lorazepam-dependent mice. The dopamine content of the cerebral cortex and thalamus and hypothalamus regions was also reduced after chronic treatment with flunarizine whereas the 5-HT content was not affected in any of the brain regions tested. Accompanying the previously mentioned effects, flunarizine significantly reduced lorazepam withdrawal symptoms in mice.

The in-vitro and in-vivo neuropharmacological actions of flunarizine documented in several publications might explain its observed effects. Thus, flunarizine has been reported to inhibit neuronal monoaminergic transmission. It inhibits potassium-stimulated monoamine release in rat striatal slices

(Godfraind 1987), and electrically induced noradrenaline release from rat cerebral cortex (Snyder & Reynolds 1985). It also inhibits the release of catecholamines from the adrenal medulla in response to nicotinic stimulation (De la Fuente et al 1992).

Flunarizine, a potent D_2 receptor antagonist (Ikegami et al 1992), attenuates the hyperactivity of dopamine neurons (Fadda et al 1989). It also enhances the decline and inactivation of dopamine in interneuronal vesicles (Nathan 1990).

It is probable that the depletion or withdrawal of lorazepam from its binding receptors in dependent mice created a pathological case in which there was an over in-flood of calcium ions (Yamamoto et al 1978), that induced a rebound activity in certain monoaminergic neurotransmitters, which was accompanied by abstinence syndrome. These effects were partially antagonized by either isradipine or flunarizine. These two drugs might be useful in reducing the intensity of lorazepam abstinence syndrome or help in the treatment of dependence.

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