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Lack of effect of nitrendipine on the pharmacokinetics and pharmacodynamics of midazolam during steady state

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1 The possible interaction (as indicated by rat experiments) between calcium channel blocking agents and benzodiazepines has been evaluated in nine healthy subjects.
2 Subsequently to an intravenous loading dose (0.07 mg kg^{-1}) midazolam was infused for 6 h (0.035 mg kg^{-1} h^{-1}) and steady state plasma levels between 54 to 114 μg l^{-1} were achieved. Two hours after the bolus of midazolam a solution of 20 mg nitrendipine or placebo was administered in a randomized, double-blind crossover fashion.
3 The marked sedative-hypnotic effects of midazolam as assessed by visual analogue scales (about four fold increase in the sedation index) and choice reaction time (100% prolongation) indicated some form of adaptation or tolerance towards the end of the infusion. However, the midazolam-induced impairments were not affected by nitrendipine.
4 EEG-data indicated stabile benzodiazepine-like effects during the complete infusion period of midazolam (e.g. decrease in alpha activity, increase in sigma, delta 2 and beta 1 activity). Again, these alterations were not modified by nitrendipine.
5 There was also no pharmacokinetic interaction between both agents, since elimination of midazolam ($t_{½}$ = 2.5 ± 0.8 h; CL = 548 ± 143 ml min^{-1}) was in close agreement with control values ($t_{½}$ = 2.4 ± 0.6 h; CL = 512 ± 102 ml min^{-1}). Likewise, plasma levels of nitrendipine were comparable to literature data.
6 Thus, it could be concluded that nitrendipine does not affect the action of midazolam and therefore a direct involvement of calcium at the benzodiazepine receptor site is unlikely under our clinical conditions.

Keywords nitrendipine midazolam pharmacokinetics pharmacodynamics drug interaction

Introduction

It is well accepted that the pharmacological actions of benzodiazepines (BZD) are mediated by γ-aminobutyric acid (GABA) and accomplished by stereoselective binding to the so-called BZD-GABA-Cl^--ionophore receptor complex (Möhler & Okada, 1977). More recently BZD-antagonists (e.g. flumazenil or CGS 8216) have been developed which by competitive binding at the receptor site specifically can block the effects of various BZD (Möhler & Richards, 1981) including midazolam (Klotz *et al.*, 1985). In a cooperative fashion BZD and GABA enhance the chloride conductance in synaptosomal membranes and it has been speculated whether other ions might be involved in the complex biochemical and electrophysiological events leading finally to anxiolyses, suppression of convulsions, muscle relaxation, sedation or sleep (Polc *et al.*, 1982).

A candidate to be considered for such involvement could be calcium. It has been recently described that $^{45}Ca^{2+}$ uptake into brain

synaptosomes was increased by BZD, especially under depolarizing conditions (Paul *et al.*, 1982; Mendelson *et al.*, 1984a). In addition, the depolarization-stimulated Ca-uptake can be blocked most effectively by the Ca-antagonist nitrendipine in rat brain synaptosomes (Turner & Goldin, 1985) and neuronal cultured cells (Freedman & Miller, 1984). Radioligand binding studies could verify that nitrendipine and other Ca-antagonists are bound to cerebral cortical membranes and these binding sites (and the corresponding Ca-channels) are mainly localized in synaptic rich zones (Gould *et al.*, 1984). In addition, tolerance to chlordiazepoxide sedation observed in mice may be mediated by calcium influx modifications (Leslie *et al.*, 1980).

This vague and indirect biochemical evidence of a theoretically possible 'interaction' between BZD and calcium or calcium-antagonists was substantiated by experiments in rats where intraventricular administration of the less potent calcium-antagonist nifedipine prevented the sleep-inducing effect of flurazepam (Mendelson *et al.*, 1984b). Besides learning more on the mode of action of BZD such an interaction could have clinical consequences since BZD and calcium-antagonists are both widely used drugs and often given at the same time.

For this reason we investigated in man whether the hypnotic effects of midazolam, as assessed by psychomotor testing and EEG-monitoring can be attenuated by the concomitant administration of nitrendipine, the most potent marketed calcium-antagonist. The study was performed under clinical relevant steady-state conditions and the protocol was almost identical to a previous study where the specific BZD-antagonist flumazenil (Ro15–1788) reversed promptly the CNS-sedation induced by an infusion of midazolam (Klotz *et al.*, 1985).

Methods

Subjects and protocol

After approval by the ethics committee of our hospital, and written informed consent was obtained, nine healthy, drug-free volunteers with a normal sleep-wake pattern (five female, four male; age range 21–35 years; weight 54 to 85 kg) participated in a placebo-controlled, double-blind, randomized crossover study.

On two occasions separated by an interval of at least 1 week the subjects received an intravenous loading dose of 0.07 mg midazolam kg^{-1} body weight followed by a constant intravenous infusion of 0.035 mg midazolam kg^{-1} h^{-1} for 6 h. A solution of placebo or 20 mg nitrendipine was ingested 2 h after the start of the infusion (under midazolam steady state condition).

Venous blood samples (10 ml) for the measurement of nitrendipine, midazolam, and its major metabolite (α-OH-midazolam) were drawn at zero time and 0.5, 1, 1.5, 2, 2.17, 2.33, 2.5, 2.75, 3, 3.5, 4, 5, 6, 7, 8, 9 and 10 h after midazolam injection. At these time points also the response to midazolam (± nitrendipine) was assessed. The baseline values were obtained prior to the midazolam bolus injection.

Measurements

Midazolam and α-OH-midazolam were measured in plasma by a specific gas-liquid chromatography (GC) assay with sensitive electron-capture detection (Allonen *et al.*, 1981; Klotz & Ziegler, 1982). Serum concentrations of nitrendipine were determined by a specific GC-MS method with minor modifications as recently described (Fischer *et al.*, 1986; during extraction procedure the charcoal purification step was omitted and a 1:1 mixture of ethylacetate/hexane was used as extraction solvent).

The sedative-hypnotic effects of midazolam were quantitatively assessed by three different methods: (1) a subjective sedation index was formed from five visual analogue scales (length each 10 cm, maximal sedative score 50 cm); (2) choice reaction time (RT1 and RT2) was measured by the Leeds psychomotor tester (mean of 10 trials).

In the choice reaction time apparatus the subject is required to scan an array of six small lights which are illuminated on a random basis. As soon as the subject detects the light (RT1) he is expected to touch the appropriate response button to extinguish the light (RT2). The latency of this response is an assessment of the integrity of the sensorimotor system and an accurate measure of psychomotor performance.

(3) EEG (central derivatives vs mastoid; C_3-A_2 position of electrodes in the 10–20-system) was monitored in supine position with closed eyes for 3 min by an EEG amplifier (time constant 0.3 s, low-pass 30 Hz). The pulse code modulated tape-stored EEG recordings were evaluated by analogue to digital conversion (250 points s^{-1}), data tapering and transformation to frequency domain by Fourier algorithm. Subsequently log power values were calculated and integrated over six epochs of 15 s; different EEG wave band ranges were selected (see Table 3). Artifacts were controlled by visual inspection.

The study was started at 08.00 h and the subjects were kept awake during the 10 h study period.

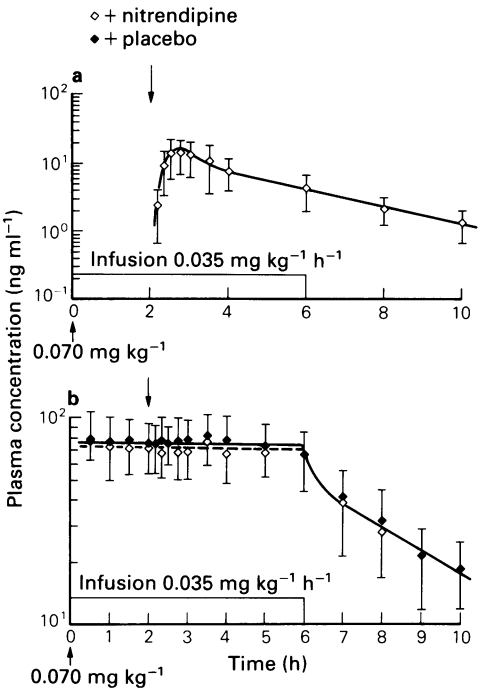

Figure 1 Plasma concentration time profiles (mean ± s.d.) of nitrendipine (a) and midazolam (b) in nine healthy subjects following an intravenous bolus/infusion regimen of midazolam and coadministration of placebo (◆) or 20 mg nitrendipine (◇), respectively.

Elimination half-lives ($t_{½}$) of midazolam and nitrendipine were individually calculated from the corresponding terminal slopes by linear regression analysis. Midazolam's total plasma clearance (CL) was derived from the ratio infusion rate/steady state concentration (C_{ss}). As no gender-related differences in the data were seen the results are expressed for all subjects as mean ± s.d. and comparison between placebo and nitrendipine were made using the paired Student's *t*-test and ANOVA.

Results

Pharmacokinetics

With the applied loading/infusion dosage regimen C_{ss} of midazolam in the range of 54–114 μg l^{-1} were rapidly achieved (Figure 1). The coadministration of nitrendipine did not change the concentration-time profile. After the stop of the infusion C_{ss} of midazolam declined with a $t_{½}$ between 1.0 and 3.7 h and CL averaged 530 ml min^{-1} (Table 1).

Absorption of nitrendipine was fast (Figure 1) as indicated by its early peak value (range 3.0 to 31 μg l^{-1}) observed within 0.5 and 1.1 h (see Table 1). Serum levels declined in the monitored time period (up to 8 h) with an apparent $t_{½}$ of 2.3 ± 0.7 h.

Pharmacodynamics

Midazolam induced an approximately twofold prolongation in RT1 and RT2, subjects felt much more tired (Table 2). In the EEG-recordings a decrease in alpha activity and

Table 1 Pharmacokinetic parameters of nitrendipine and midazolam in nine healthy subjects

	Nitrendipine			*Midazolam*					
	C_{max}	t_{max}	$t_{½}$	C_{ss} (μg l^{-1})		CL (ml min^{-1})		$t_{½}$ (h)	
Subject	(μg l^{-1})	(h)	(h)	+P	+N	+P	+N	+P	+N
1	3.0	0.67	2.5	86	64	579	775	2.1	1.0
2	16.6	0.77	2.2	63	66	503	480	1.8	2.2
3	18.5	0.59	2.0	101	114	364	323	2.1	2.4
4	16.0	0.75	2.0	57	54	624	662	2.8	3.7
5	30.9	0.82	1.7	65	56	519	601	2.7	7.0*
6	13.7	0.93	1.9	69	66	521	550	1.8	2.6
7	11.7	0.50	4.1	57	56	660	667	2.6	3.3
8	11.8	1.15	2.2	97	92	392	411	2.2	2.0
9	10.4	0.21	1.8	72	68	435	461	2.8	2.9
Mean	14.7	0.71	2.3	72	71	512	548	2.4	2.5
s.d.	7.6	0.27	0.7	18	20	102	143	0.6	0.8

* Excluded from the calculation of the mean.
N nitrendipine, P placebo.

Table 2 Psychometric measurements of the sedative-hypnotic action of midazolam in nine healthy subjects at three different time points

	RT1 (ms)						*RT 2 (ms)*						*Sedation index (cm)*					
	Baseline		*Midazolam ss at C_{max} of N*				*Baseline*		*Midazolam ss at C_{max} of N*				*Baseline*		*Midazolam ss at C_{max} of N*			
Subject	*+P*	*+N*	*+P*	*+N*	*+P*[e]	*+N*[e]	*+P*	*+N*	*+P*	*+N*	*+P*[e]	*+N*[e]	*+P*	*+N*	*+P*	*+N*	*+P*[e]	*+N*[e]
1	216	212	532	281	268	301	409	475	780	621	432	535	10.7	13.6	31.4	23.0	18.9	22.7
2	303	320	754	664	680	604	425	445	865	839	970	845	11.7	10.4	42.1	35.4	37.9	33.4
3	215	251	402	416	307	467	282	346	515	550	427	543	9.7	11.2	44.7	26.6	43.9	23.5
4	330	287	865	684	352	339	448	384	1097	684	461	448	18.8	6.0	30.3	18.7	22.2	13.6
5	335	298	516	394	516	358	430	425	704	597	653	558	2.8	9.4	41.8	36.7	35.2	38.6
6	313	316	424	413	365	530	427	422	821	645	504	722	0.4	5.0	44.7	39.4	41.4	38.3
7	298	287	894	640	380	858	370	393	1268	803	502	929	7.3	15.7	30.5	46.9	20.0	40.4
8	316	245	496	780	403	444	389	330	704	865	542	623	8.3	5.8	34.7	32.0	31.4	24.0
9	345	340	596	424	380	413	417	440	691	573	575	544	2.9	2.4	24.1	41.1	22.0	35.7
Mean	297	284	609[a]	493[b]	406[c]	479	400	384	827[a]	686[a]	563[c]	639	8.1	8.8	36.0[a]	33.3[a]	30.3[d]	30.0[c]
s.d.	48	41	184	162	123	171	50	71	228	119	169	160	5.6	4.4	7.5	9.1	9.8	9.3

ss: during steady state conditions of midazolam prior to the coadministration of nitrendipine or placebo, respectively.
N nitrendipine, P placebo.
[a] $P < 0.001$ (*vs* corresponding baseline value) two-sided.
[b] $P < 0.005$ (*vs* corresponding baseline value) paired *t*-test.
[c] $P < 0.05$ (*vs* corresponding midazolam ss value) (following ANOVA).
[d] $P < 0.005$ (*vs* corresponding midazolam ss value).
[e] no difference placebo *vs* verum.

Table 3 EEG-data (mean ± s.d.) of nine healthy subjects during the midazolam-nitrendipine interaction study

Frequency band (Hz)				*Baseline* +P	*Baseline* +N	*Midazolam ss* +P	*Midazolam ss* +N	*at* C_{max} *of N* +P[f]	*at* C_{max} *of N* +N[f]
$Delta_2$	1.5	to	3.5 Hz	19.8 ± 6.8	22.5 ± 9.2	28.7 ± 7.9[a]	28.4 ± 6.8[b]	24.6 ± 8.3[c]	26.5 ± 7.3
Theta	3.5	to	7.5 Hz	21.8 ± 4.0	22.4 ± 5.6	22.0 ± 4.6	21.8 ± 3.4	21.1 ± 4.8	19.9 ± 3.4
Alpha	7.5	to	12.5 Hz	33.6 ± 14.5	33.9 ± 12.6	18.3 ± 6.2[a]	17.8 ± 4.3[a]	21.6 ± 10.4	21.0 ± 6.3
Sigma	12.5	to	14.5 Hz	4.8 ± 2.1	4.7 ± 1.8	9.3 ± 1.6[d]	8.9 ± 3.3[e]	8.1 ± 2.6	8.9 ± 4.7
$Beta_1$	12.5	to	20 Hz	14.4 ± 6.0	13.1 ± 4.5	24.6 ± 6.9[d]	23.9 ± 5.7[d]	25.0 ± 8.1	24.2 ± 5.7

ss: during steady state conditions of midazolam prior to the coadministration of nitrendipine or placebo, respectively.
N nitrendipine, P placebo.
[a] $P < 0.005$ (*vs* corresponding baseline value) two-sided.
[b] $P < 0.01$ (*vs* corresponding baseline value) paired *t*-test.
[c] $P < 0.01$ (*vs* corresponding midazolam ss value) (following ANOVA).
[d] $P < 0.01$ (*vs* corresponding baseline value).
[e] $P < 0.05$ (*vs* corresponding baseline value).
[f] no difference placebo *vs* verum.

increases in delta and beta activity could be observed (Table 3). While the alterations in the EEG remained fairly constant during the midazolam infusion (Figure 2), sedation index and especially RT1/RT2 indicated less deterioration towards the end of the infusion compared to the beginning of the infusion (Figure 3) despite C^{ss} of midazolam remained stable (Figure 1). After stopping the midazolam infusion all pharmacodynamic alterations returned to baseline levels within approximately 2 h (Figures 2 and 3).

Following the administration of nitrendipine no significant attenuation modifications of the sedative-hypnotic action of midazolam — as assessed by all three pharmacodynamic tests — could be substantiated (Tables 2 and 3 and Figures 2 and 3).

Discussion

From our interaction study it is obvious that the CNS-depressant effects induced by midazolam are of short duration, since this benzodiazepine is rapidly eliminated (Figure 1). The pharmacokinetic parameters (e.g. $t_{½}$, CL) calculated from this steady state experiment (see Table 1) are in close agreement to data derived from single dose studies (Klotz & Ziegler, 1982; Kanto, 1985). Thus, a linear pharmakokinetic behaviour can be assumed. In addition, the disposition of midazolam was not affected by the coadministration of nitrendipine and consequently a pharmacokinetic interaction can be excluded.

Following a single intravenous or oral dose of midazolam good relationships between the plasma levels and the pharmacodynamic response of midazolam have been evaluated (Alonen *et al.*, 1981). In general, this seems to be true also during steady state conditions (Figures 2 and 3). However, it appears that the sedative effects as assessed by the subjective sedation index and the objective measurement of the choice reaction time are attenuated towards the end of the midazolam infusion (Figure 3) indicating some form of acute adaptation or tolerance. Such a trend to normalized test values was already previously observed when midazolam was infused for 26 h (Klotz & Reimann, 1984). A pure learning effect to the applied tests is unlikely because the subjects had been trained prior to the study until stabile baseline levels were achieved. During the prolonged infusion of midazolam a functional adaptation (e.g. down regulation of benzodiazepine receptors) could occur and/or a learned tolerance might be operating. A similar form of tolerance to the self-ratings of sedative effects has been observed after 2 weeks of dosing with diazepam (Ochs *et al.*, 1983). However, the EEG as a more sensitive and objective pharmacodynamic assessment gave no evidence of any acute tolerance phenomena (Figure 2). This would suggest that self-rating scales and choice reaction time are of limited value, particularly for repeated testing, to characterize the present status of the CNS and that long-term sedative-hypnotic drug effects should be monitored by the EEG. It is also feasible that psychomotor tests and EEG measure different CNS-activities or functions.

Extrapolation of biochemical *in vitro* experiments (Paul *et al.*, 1982; Mendelson *et al.*, 1984a) and an animal study (Mendelson *et al.*, 1984b) could suggest that the action of benzodiazepines

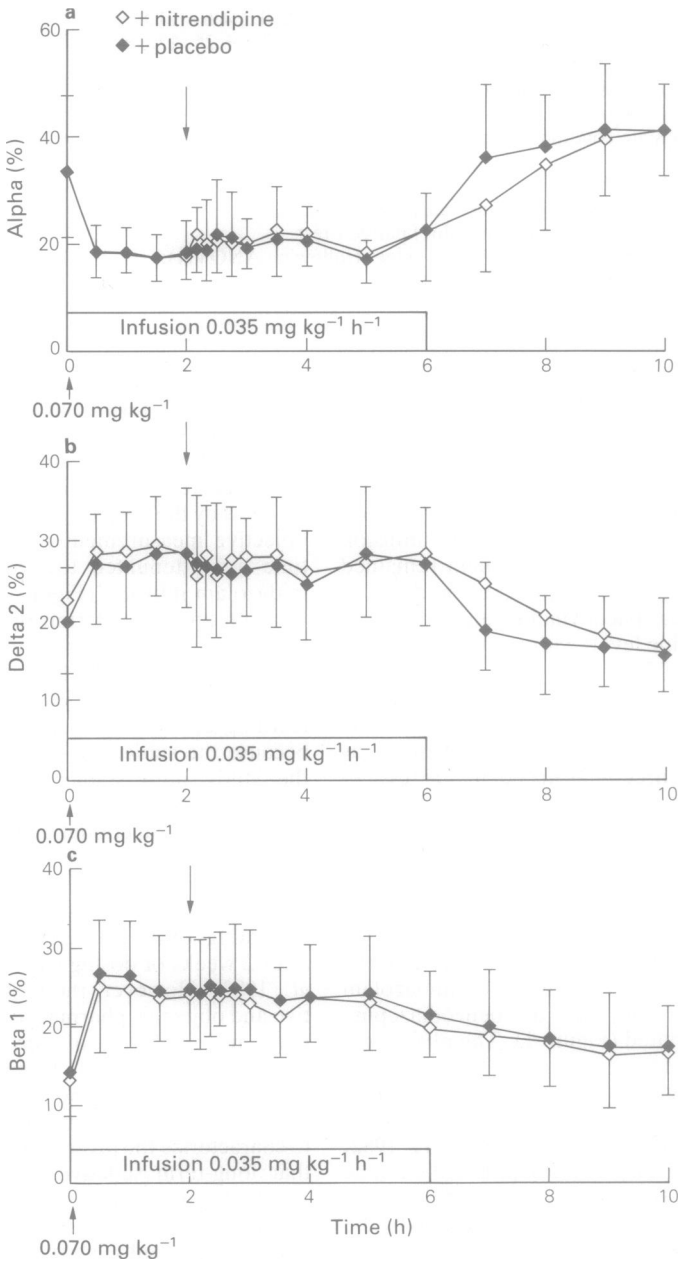

Figure 2 Time course of the monitored EEG-activity during the midazolam-nitrendipine interaction study in nine healthy subjects; percentages of alpha-activity (a), delta$_2$-activity (b) and beta$_1$-activity (c) are given as mean ± s.d. ◆ + placebo, ◇ + nitrenidipine.

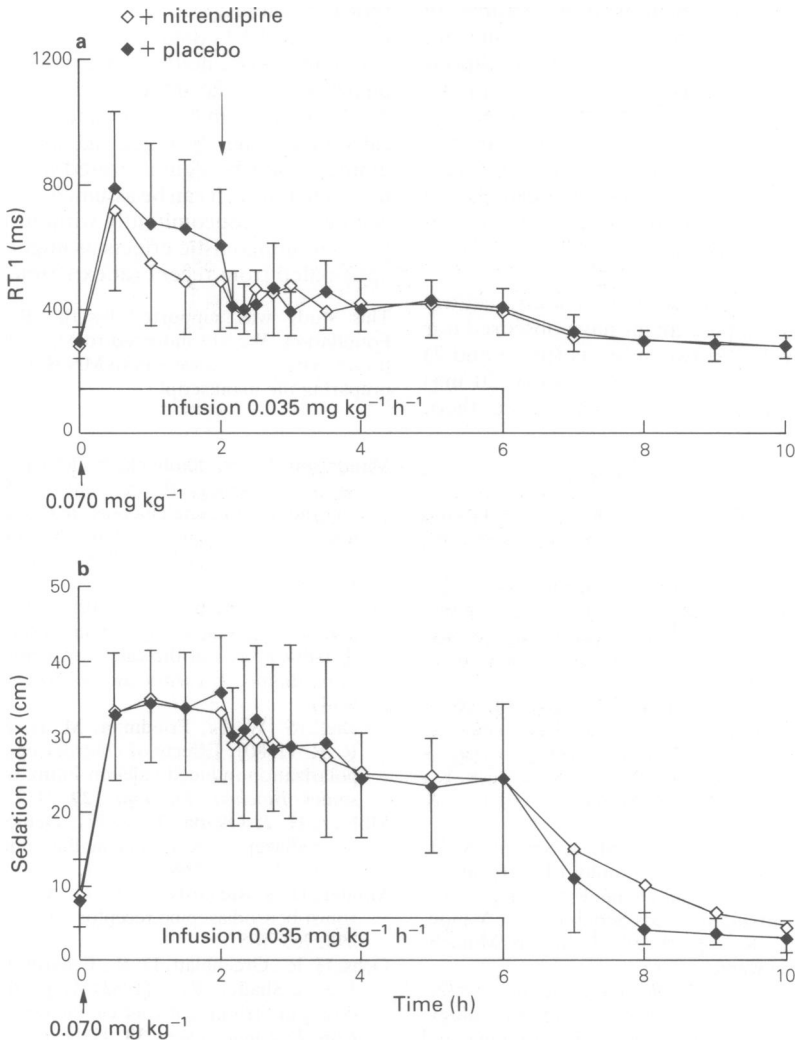

Figure 3 Time course (mean ± s.d.) of the sedation index (b) and the choice reaction time RT1 (a) during the midazolam interaction study with coadministration of nitrendipine (◇) or placebo (◆) in nine healthy subjects.

might be antagonized by calcium channel blocking agents. We used the potent nitrendipine to test whether such interaction occurs also in man, which would be of clinical and theoretical relevance. The applied dose (20 mg in solution) resulted in plasma levels (Figure 1) which can be regarded as effective in terms of inducing cardiovascular effects (Mikus & Eichelbaum, 1987). During all our studies the monitored blood pressure decreased maximally 5 to 10 mg Hg. All three test systems (Figures 2 and 3 and Tables 2 and 3) demonstrated clearly that nitrendipine was not able to alter significantly the response to midazolam.

Thus with the dose applied and under our experimental design no pharmacodynamic interaction was visible. Consequently under clinical conditions changes in calcium channel function seems to be not involved in the sedative-hypnotic action of benzodiazepines. The obvious discrepancy to the above mentioned rat experiments (Mendelson *et al.*, 1984b) might be due to — besides species differences — the route of administration of the calcium channel antagonist. In the animal experiments nifedipine (20 $\mu g\ kg^{-1}$) was injected intraventricularly whereas our subjects ingested a solution of 20 mg nitrendipine. This clinically recommended dose will probably not result in such high brain levels as achieved by the direct CNS-administra-

tion of nifedipine which is only feasible in animals. Therefore our study cannot rule out that under certain experimental conditions calcium might be involved somewhere in the chain of events between binding of benzodiazepines at their recognition site on the GABA-receptor complex and expressing their pharmacological effects. However, under clinical conditions an antagonistic effect of calcium channel blocking agents on the action of benzodiazepines is very unlikely in man.

To exclude the likelihood that any central effects of nitrendipine might have obscured our results, we tested in two subjects (no. 5 and 7) whether nitrendipine (oral solution of 20 mg) induced any EEG-changes. This more theoretical possibility could not be substantiated by the derived EEG data.

In conclusion, neither a pharmacokinetic nor pharmacodynamic interaction between benzodiazepines (e.g. midazolam) and dihydropyridine calcium channel blocking agents (e.g. nitrendipine) could be demonstrated in healthy subjects. Therefore, it can be assumed that both drugs can be given concomitantly without taking into account antagonistic effects as might have been anticipated from recent rat experiments.

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