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Free Fatty Acids, Central Nervous System and Bromazepam

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Summary: The concentrations of free fatty acids in plasma were estimated in thirty-three subjects, before and after (24 hours and 72 hours) the start of bromazepam oral administration in a daily dose of 6 mg (1.5 mg at 7 a.m., 1.5 mg at 3 p.m. and 3 mg at 11 p.m.). A fourth estimation was performed 72 hours after drug discontinuation. A control group of twelve subjects received a placebo according to the same dosage pattern.

In comparison with the first estimation, the subsequent free fatty acid values showed a significant decrease ($p < 0.05$, $p < 0.05$ and $p < 0.001$, respectively), whereas the control group showed no significant decrease ($p > 0.05$).

The aim of the present article is to present the results of the three day-standard oral administration of bromazepam on free fatty acid concentration of plasma.

All the samples, including the control group, were taken from a population free from any disease affecting adipose tissue metabolism.

Freie Fettsäuren, Zentralsnervensystem und Bromazepam

Zusammenfassung: Bei 33 Personen wurden die freien Fettsäuren im Plasma vor und nach Beginn (24 h und 72 h) der oralen Verabreichung von 6 mg Bromazepam pro die bestimmt. 72 Stunden nach dem Absetzen des Medikaments wurde die Konzentration der freien Fettsäuren erneut bestimmt. Diese Bestimmung nach demselben Schema erfolgte auch bei einer Kontrollgruppe von 12 Probanden, die ein Placebo erhielten.

Im Vergleich zum Resultat der ersten Bestimmung zeigen die nachfolgenden Werte eine signifikante Verminderung. Hingegen wurde bei der Kontrollgruppe keine Abnahme festgestellt.

Zweck dieser Arbeit war die Analyse der Einwirkung von Bromazepam auf die freien Fettsäuren im Plasma nach dreitägiger standardisierter oraler Verabreichung des Medikaments. Die Probanden, sowohl die Patienten wie auch die Kontrollpersonen, wiesen keine Krankheit auf, die den Stoffwechsel des Fettgewebes beeinflusst.

Introduction

The influence of the central nervous system (CNS) on lipid metabolism and particularly, on free fatty acid mobilization is indisputable. Its exact mechanism, however, remains to be clarified.

The concentration of free fatty acids in plasma is generally accepted as a sensitive measure of CNS arousal (1-6).

The purpose of the present study is to examine the possible influence of a known CNS-acting drug, the tranquillizer bromazepam, on the concentration of free fatty acids in human plasma. Such an influence would

constitute indirect evidence of CNS regulation of the mobilization of free fatty acids in man, mainly through the hypothalamus and limbic system.

Materials and Methods

Patients

Thirty-three subjects, hospitalized for a clinical and laboratory investigation, were selected for the apparent absence of any organic disease; this disease-free status was later confirmed. To minimise any possible distress from the hospitalization itself, the subjects were studied between the 2nd and 3rd day after admission. All of them were in a 12-hour state of fasting during the

four morning periods of observation. There was no tobacco, coffee or tea consumption. No subject was receiving medication, and all subjects were resting without exercise. The women, still in reproductive age, were studied between the 10th and 20th day of their menstrual cycle.

The venipunctures were all performed by the same physician, when the patient was still in bed, just after awakening, and the tourniquet application did not exceed 60 seconds. The administered tranquillizer was the benzodiazepine, bromazepam (Lexotan® (Roche)), administered orally.

Four samples were taken from each subject, namely:

- I. Before drug administration.
- II. 24 hours after the first administration of the drug (6 mg/24 h in doses of 1.5 mg at 7 a.m., 1.5 mg at 3 p.m. and 3 mg at 11 p.m.).
- III. 72 hours after the first administration of the drug, with the same dosage pattern as above.
- IV. 72 hours after drug discontinuation.

It was not possible to complete the study for all subjects, because some of them left the hospital earlier than anticipated.

Another group of subjects (12 cases) was used as a control group. These subjects, which were selected under the same conditions, were given placebo tablets according to the above dosage pattern.

Method

Blood was collected into an heparinized syringe at the various time intervals stated above, cooled at 4 °C, and centrifuged for 10 min at 3000 min⁻¹. The plasma was separated immediately after collection and deep frozen at -20 °C for later analysis. Plasma free fatty acids were estimated in duplicate by the method of *Ducombe* using 0.2 ml of plasma (7). For this purpose the reagent kit No 126055 of Boehringer Mannheim GmbH and palmitic acid standard were used. The quality of the assay was estimated by measuring 10 replicate samples of two plasma pools (0.16 and 0.38 mmol/l). In accordance with a previous report (8) the intraassay coefficient of variation was lower than 5.0%. All samples of each patient were determined together in the same run immediately after the end of the test period. The results are given as the mean of duplicate samples. Statistical comparison between the four samples of each subject was made by the t-pair test.

Results

Comparison between values I and II (tab. 1)

The free fatty acid concentrations in the first samples (before drug administration) ranged from 125 µmol/l to 815 µmol/l plasma (mean value 422 ± 29.5 SEM µmol/l), i.e. within normal limits, except for five cases which were a little above the upper limit.

Values for the free fatty acid concentration of the second samples (24 h after beginning of drug administration) ranged from 110 µmol/l to 780 µmol/l plasma (mean value 363 ± 25 SEM µmol/l), i.e. within normal limits, only one case being slightly above the upper limit.

Values for the second samples were found to be significantly lower than those for the first samples ($p < 0.05$ using the t-pair statistical analysis). In 24 out of 33 cases

Tab. 1. Free fatty acid concentration values (µmol/l plasma) of patients before (I) and 24 h after the first drug administration (II) and relative changes (33 cases).

Case	Free fatty acids (µmol/l)		Change (%)
	I	II*	
1	330	221	- 33.6
2	505	441	- 12.7
3	470	294	- 37.4
4	345	218	- 36.8
5	300	175	- 58.3
6	349	297	- 14.9
7	230	490	+ 112.7
8	125	110	- 11.2
9	225	240	+ 6.6
10	249	122	- 51.0
11	136	157	+ 15.4
12	368	357	- 2.9
13	475	429	- 9.7
14	440	455	+ 3.4
15	567	581	+ 2.4
16	620	468	- 24.5
17	815	780	- 4.3
18	492	439	- 10.7
19	510	255	- 50.0
20	363	504	+ 38.8
21	614	467	- 23.9
22	576	423	- 26.6
23	581	454	- 21.9
24	576	527	- 8.5
25	400	205	- 48.8
26	278	256	- 8.0
27	317	371	+ 17.0
28	337	329	+ 15.1
29	671	428	- 36.2
30	775	580	- 20.0
31	378	346	- 8.5
32	298	334	+ 12.8
33	238	215	- 9.7

* $p < 0.05$ (t-pair test statistical analysis of the II versus the I estimation).

the values obtained were decreased from 2.9% to 58.3% with a mean value of 23.7 ± 3.4 SEM %.

In three cases a small increase was noted (mean value: 4.13 ± 1.27 SEM %). In four cases an increase was noted (mean value: 14.9 ± 1.0 SEM %), whereas in two extreme cases rather large increases were noted (38% and 112.7%).

Comparison between values I and III (tab. 2)

In 29 cases out of the initial 33, where a third sample was available (72 hours after initiation of drug administration), the free fatty acid concentrations were also within normal limits, ranging between 98 µmol/l and 808 µmol/l plasma (mean value: 353 ± 31 SEM µmol/l). Three cases were a little higher. In all 29 cases the free fatty acid concentrations in the plasma were significantly lower ($p < 0.05$) in III than in I, using the t-pair test.

In 20 cases the observed decrease ranged from 9.1% to 67.1% (mean value: 27.8 ± 2.8 SEM %), in four cases

Tab. 2. Free fatty acid concentration values ($\mu\text{mol/l}$ plasma) of patients before (I) and 72 h after the first drug administration (III) and relative changes (25 cases).

Case	Free fatty acids ($\mu\text{mol/l}$)		Change (%)
	I	III*	
2	505	352	- 30.3
3	470	199	- 57.6
4	345	337	- 2.3
5	300	280	- 6.6
6	349	206	- 40.9
10	249	137	- 44.9
11	136	111	- 15.4
12	368	199	- 45.9
14	440	440	0
15	567	577	+ 1.7
16	620	496	- 20.0
17	815	446	- 45.2
20	363	318	- 12.4
22	576	434	- 24.6
23	581	368	- 36.6
24	576	467	- 18.3
25	400	338	- 15.5
26	278	216	- 22.3
27	317	243	- 23.3
28	337	286	- 15.1
29	671	277	- 58.7
30	775	786	- 0.9
31	378	326	- 13.7
32	298	344	+ 15.4
33	238	252	+ 5.9

* $p < 0.05$ (t-pair test statistical analysis of the III versus the I estimation)

Tab. 3. Free fatty acid concentration values ($\mu\text{mol/l}$ plasma) of patients before (I) and 72 h after drug discontinuation (IV) and relative changes (29 cases).

Case	Free fatty acids ($\mu\text{mol/l}$)		Change (%)
	I	IV*	
2	505	382	- 24.3
3	470	270	- 42.5
4	345	301	- 12.8
5	300	235	- 21.6
6	349	253	- 27.5
7	230	171	- 35.7
9	225	175	- 22.2
10	249	98	- 67.1
11	136	147	+ 8.0
12	368	294	- 20.1
13	475	514	+ 8.2
14	440	400	- 9.1
15	567	602	+ 6.1
16	620	734	+ 18.3
17	815	514	- 36.9
18	492	563	+ 14.4
20	363	390	+ 7.4
22	576	399	- 30.7
23	581	427	- 26.5
24	576	684	+ 18.7
25	400	291	- 27.2
26	278	210	- 24.5
27	317	166	- 33.8
28	337	270	- 19.8
29	671	808	+ 20.4
30	775	519	- 26.2
31	378	333	- 11.9
32	298	192	- 35.5
33	238	288	+ 21.0

* $p < 0.001$ (t-pair statistical analysis of the IV versus the I estimation).

a small increase was observed (mean value: 7.4 ± 0.47 SEM %), and in five more cases a moderate increase was observed (mean value: 18.6 ± 1.15 SEM %).

Comparison between I and IV values (tab. 3)

In 25 out of 33 cases, where a fourth sample was available (72 hours after drug discontinuation), the values obtained ranged between $115 \mu\text{mol/l}$ and $786 \mu\text{mol/l}$ (mean value: 387 ± 28 SEM $\mu\text{mol/l}$), that is, within normal limits except for one which was a little above the upper normal limit. In that group too, there was a significant decrease (t-pair test) of the values, compared with the first determination ($p < 0.001$).

The values obtained decreased in 21 cases, ranging from 0.9% to 58.7% (mean value: 26.2 ± 3.7 SEM %); in one case there was no difference and in three cases the observed increase had a mean value of 7.66 ± 4.0 SEM %.

In 12 members of the control group (tab. 4) the free fatty acid concentrations ranged from 201 to $472 \mu\text{mol/l}$ plasma (mean: 315 ± 26 SEM $\mu\text{mol/l}$), from 210 to $699 \mu\text{mol/l}$ (mean: 378 ± 42 SEM $\mu\text{mol/l}$) from 268 to $635 \mu\text{mol/l}$ (mean: 392 ± 37 SEM $\mu\text{mol/l}$) and from

212 to $774 \mu\text{mol/l}$ (mean: 414 ± 65 SEM $\mu\text{mol/l}$) for the I, II, III and IV measurements, respectively.

The observed changes between the I and the II, III and IV measurements were revealed by the use of the t-pair test ($p > 0.05$, $p > 0.05$, $p > 0.05$, respectively).

Analytically, on 9 out of the 12 members of the control group, the free fatty acid concentrations showed a percentage increase between the first and the second estimation ranging from 5.5% to 77.2% (mean value: 36.9 ± 9.4 SEM %) while in the remaining 3 cases the values were decreased by 9.7% to 39.7% (mean value: 22.4 ± 8.9 SEM %).

In 9 out of the 12 cases the values between the first and the third measurement were increased (5.8% to 91.1%, mean value: 44.6 ± 9.4 SEM %) and in 3 cases decreased (4.9% to 20.4%, mean value: 13.2 ± 4.5 SEM %).

In 10 out of the 12 members in the same control group the values obtained between the first and the fourth estimation were increased (4.2% to 91.3%, mean value: 45.8 ± 9.3 SEM %) and in 2 cases decreased (7.2 to 43.8%, mean value 25.5 ± 18.2 SEM %).

Tab. 4 Free fatty acid concentration values ($\mu\text{mol/l}$ plasma) of patients before (I), 24 h (II), 72 h (III) after the first drug administration and 72 h after drug discontinuation (IV) and relative changes in control group (12 cases).

Case	Free fatty acids ($\mu\text{mol/l}$)			Free fatty acids ($\mu\text{mol/l}$)			Free fatty acids ($\mu\text{mol/l}$)		
	I	II*	Change (%)	I	III**	Change (%)	I	IV***	Change (%)
1	351	211	-39.7	351	279	-20.4	351	393	+11.9
2	241	325	+34.7	241	415	+72.0	241	370	+53.4
3	442	521	+17.8	442	468	+5.8	442	410	-7.2
4	378	341	-9.7	378	406	+7.3	378	212	-43.8
5	282	369	+30.7	282	369	+30.7	282	322	+14.1
6	201	336	+67.0	201	302	+50.1	201	268	+33.2
7	472	529	+12.0	472	404	-14.3	472	706	+49.3
8	404	699	+72.8	404	635	+57.0	404	774	+91.3
9	272	287	+5.5	272	352	+29.4	272	410	+50.7
10	256	210	-17.9	256	392	+53.1	256	434	+75.4
11	226	259	+14.5	226	432	+91.1	226	398	+76.1
12	259	459	+72.2	259	351	+35.5	259	270	+4.2

* $p > 0.05$ (t-pair test statistical analysis of the II versus I estimation)

** $p > 0.05$ (t-pair test statistical analysis of the III versus I estimation)

*** $p > 0.05$ (t-pair test statistical analysis of the IV versus I estimation).

All cases under drug administration were classified according to the fluctuations of the values obtained, as follows:

Group A: This includes 18 cases, where all the values obtained after drug administration were always lower (fig. 1) than the first one.

Group B: 7 cases where the observed values were increased in estimation II and then decreased (fig. 2). In 5 cases the decrease was in estimation III, and in 2 cases in estimation IV. In other words, the free fatty acid values of this group also eventually show a decrease.

Group C: This comprises 6 cases where the decrease in estimation II is followed by an increase in estimation III (fig. 3).

In 3 of them there is a decrease in estimation IV with respect to estimation I. In 2 of them there was no IV estimation, and in only one case all the values remained at slightly (5.9%) higher levels.

Group D: This includes only 1 case (fig. 4) where II and III were decreased, followed by a slightly increased (1.2%) IV.

Group E: The only included case (fig. 5) gave slightly increased values in all estimations (2.4%, 6.1% and 1.7%, respectively).

Briefly, in 18 out of 33 cases the concentration of free fatty acids in plasma were decreased in all estimations, under drug influence. In 14 out of the remaining 15 cases there was a decrease in one or two estimations, and in one case only all the values were a little increased with respect to estimation I.

In contrast, there is no statistical correlation ($p > 0.05$) between the obtained values in the control group, after placebo administration (fig. 6).

Discussion

The results obtained, in all but one case, show a statistically significant decrease ($p < 0.05$ to $p < 0.001$) of the concentrations of free fatty acids in plasma, with respect to those before treatment.

The above-mentioned decrease in concentrations of free fatty acids in plasma has the characteristic of being observed after 24 h administration and persists after the 72 hours of administration ($p < 0.05$) as well as 72 hours after bromazepam discontinuation ($p < 0.001$). A lot of research work, during the last years, refers to the CNS influence on the mobilization of free fatty acids (1-5, 9). The exact mechanism of such an influence is still unsettled, since the clinical studies as well as the experimental ones have led to various hypotheses as to the direct or indirect influence of the CNS on lipid metabolism. It has been shown that electrical stimulation of the hypothalamus causes a prompt elevation of the concentration of free fatty acids in plasma (10). Sapira et al. (11) found significant elevation in plasma free fatty acids, after psychological stimulation in volunteers. It is of interest to emphasize the gradual diminution of this reaction after the adaptation to the stimulus (12). Similar studies, clinical or experimental, show that any CNS stimulation, physical (9, 10, 13) or psychological (1, 4, 14), results in plasma free fatty acid elevation.

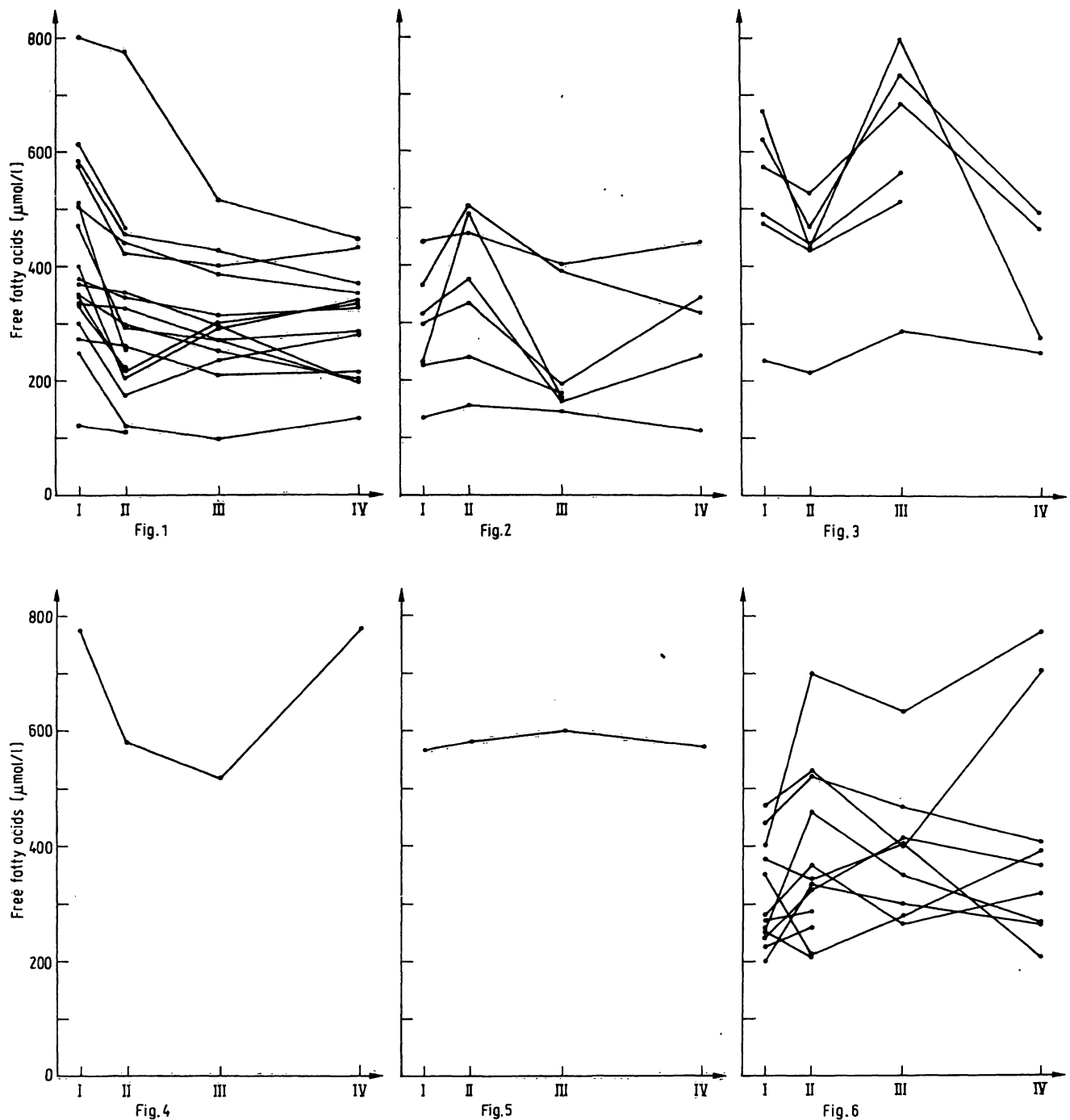


Fig. 1-6. Classification of all cases according to the fluctuations of the obtained free fatty acid concentration values.

- I = Before drug administration
- II = 24 h after first drug administration
- III = 72 h after first drug administration
- IV = 72 h after drug discontinuation.

On the other hand, peripheral blockade of the sympathetic "autonomic" nervous system by means of adrenergic or ganglionic blocking agents, inhibits the physiologic response of increased free fatty acids after threatening stimuli (5, 15-18). Experimental studies on animals prove the necessity of the peripheral nerve integrity in order to maintain an equilibrium in lipid metabolism (19).

This is probably due to the function of the autonomic routes of the nerve. Therefore, in case of nerve section, transmission of efferent autonomic impulses, chiefly from the hypothalamus, is interrupted.

It is generally accepted that the action of the nervous system upon free fatty acids is exerted by means of a complicated humoral mechanism (20). This includes

a considerable number of hormones and peptides, which contribute in maintaining a complex dynamic equilibrium, by either enhancing or inhibiting each other in various proportions (21). The substances known to be active in lipid mobilization, from adipose tissue, include adrenal steroids, glucagon, somatostatin, endorphins and enkephalins (22) and the hypophyseal hormones vasopressin, thyrotropin, corticotropin, prolactin, somatotropin and the β - and γ -lipotropins (20), but the most important hormones regulating lipolysis are the neural and circulating catecholamines. The increase of catecholamines results in the elevation of the concentration of free fatty acids in plasma. The main pathway of catecholamine action on lipolysis is believed to be through the activation of adenylate cyclase, leading to an increased level of cAMP, which leads to phosphorylation and activation of triglyceride lipase. It seems likely that other pathways of catecholamine lipolysis, non cAMP-dependent, may also exist but their exact action remains unclear as yet (23). On the other hand, it is well-known that the catecholamine plasma concentration is elevated during the state of "stress" which results from the arousal of CNS (17).

Thus the concentration of free fatty acids in plasma expresses one of the biochemical parameters of "stress".

Many investigators (9, 24–27) have worked on the response of free fatty acid concentrations in plasma to the administration of tranquillizers acting on the CNS. These studies were either performed on experimental animals or the tranquillizer was administered after induced hyperlipidaemia (26) or experimental neural stimulation. In these studies the estimation of free fatty acids was performed only after a single dose of the drug under investigation. Thus a

comparison of our results with those concerning other benzodiazepines is not possible, because in the present study the administration of the tranquillizer is constant and of a long duration, imitating the usual clinical administration. This kind of administration plus the pharmacokinetic properties of bromazepam (half-life time 21–24 h, regular elimination due to the absence of other active metabolites) (28) limits the role of inter-individual variations of plasma bromazepam concentrations. In addition, the timing of estimations of free fatty acid concentration in plasma, as already described, examines both the cumulative phase and the steady state of bromazepam plasma concentration. Throughout this "steady state" phase intrasubject fluctuations were limited to a large extent.

The presence of active bromazepam plasma concentrations during the drug administration was judged by the clinical appearance of the patients i.e. their behavioural arousal level. They were slightly somnolent, whereas before the first dose they were normally awake. Finally the opinion that benzodiazepines exert a direct action on the releasing rate of free fatty acids from adipose tissue is excluded by the results of in vitro studies (9). We consider that our findings are more relevant to the true action of the drug, because the significant decrease of free fatty acids was observed in individuals under normal conditions, without a previous deviation of their neural or metabolic functions. Furthermore, continuous drug administration represents a more constant action on the concentration of free fatty acids in plasma and is closer to the routine practice, where the tranquillizer is not administered in a single dose. In conclusion, our findings constitute one more indication that CNS inhibition causes a decisive control over lipid mobilization, reducing the concentration of free fatty acids in plasma.

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