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Behavioral and electroencephalographic effects of chronic administration of diazepam in rats and rabbits

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Summary. – *In order to study the electroencephalographic (EEG) and behavioral effects of chronic administration of benzodiazepines, rats were treated daily for 10 days with 10 mg/kg i.v. of diazepam; rabbits were treated daily for 12 days with 20 mg/kg i.v. The sedative effect disappeared between the 3rd day in rats and the 10th day in rabbits of treatment, when a syndrome characterized by slight motor excitation, compulsive gnawing and eating emerged. This syndrome was accompanied by the appearance of 20-30 c/s waves (beta-like activity) in the EEG recordings, reaching the plateau at the 6th day of treatment in rats and at the 9th day of treatment in rabbits.*

This pattern was still present upon discontinuation of the drug and lasted up to the 15th day after the end of treatment. During this withdrawal period, the administration of a dose of 0.7 mg/kg i.v. of diazepam elicited an excitatory syndrome accompanied by increase of the beta-like activity in the EEG. This was blocked by administration of naloxone (5 mg/kg i.v.).

Sommario. – *Uno studio elettroencefalografico e comportamentale è stato eseguito dopo somministrazione ripetuta di diazepam nei ratti (10 mg/kg i.v. per 10 giorni) e nei conigli (20 mg/kg i.v. per 12 giorni). L'effetto sedativo del diazepam scompare dopo il terzo giorno di trattamento nei ratti e dopo il decimo nei conigli, allorché subito dopo l'assunzione del farmaco insorge una sindrome caratterizzata da una leggera eccitazione, masticazione ed assunzione di cibo. Questa sindrome è accompagnata, dal punto di vista EEGrafico, dalla comparsa di onde a basso voltaggio di 20-30 c/s (attività beta-simile). Tale andamento EEGrafico raggiunge la massima durata al sesto giorno di trattamento nei ratti ed al nono nei conigli, ed è presente dopo sospensione del trattamento per altri quindici giorni. Durante questo periodo di astinenza, la somministrazione di 0.7 mg/kg i.v. di diazepam provoca nei ratti la ricomparsa di tale sindrome eccitatoria, accompagnata dalla presenza di attività beta-simile sul tracciato EEGrafico. Tale attività beta-simile viene bloccata dalla somministrazione di naloxone (5mg/kg i.v.).*

INTRODUCTION.

Benzodiazepine derivatives belong to the class of « minor tranquillizer »; due to their weaker toxicity these drugs superseded the barbiturate and meprobramate prescriptions and achieved a widespread use in the clinic as anxiolitic, sedative, anticonvulsant and miorelaxant drugs.

Although it is known that benzodiazepines have few side effects, there are clinical and experimental reports which indicate that administration of high doses of these drugs, over a prolonged period of time, might produce adverse effects (Tab. 1). Under benzodiazepine treatment, tolerance to their sedative and anticonvulsant [1–4] actions was reported.

Withdrawal signs upon abrupt discontinuation of the drug were also described [1–5] as well as the „ Floppy infant syndrome " (Tab. 1), reported in newborns of mothers who took large doses of benzodiazepines during pregnancy [6–8].

Table 1. – *Adverse effects due to the chronic administration of benzodiazepines*

1) Tolerance to	Sedative effect [18] Anticonvulsivant effect [2]
2) Withdrawal signs	Anxiety [3] « Rebound Insomnia » [5]
3) „ Floppy Infant syndrome " [6–8]	Muscular Hypothonia Sucking Difficulties Hypothermia Attacks of Cyanosis

The discovery of benzodiazepine specific recognition sites [9], functionally linked to the GABA receptors [10–13] in animal and human brain opened a new avenue particularly for the study of the tolerance and withdrawal effects due to the chronic administration of benzodiazepines. Experimental studies showed no significant modifications of the binding characteristics of both GABA and benzodiazepines during chronic treatment. However, in newborn rats of dams which received high doses of diazepam throughout pregnancy the capability of muscimol in stimulating ^{3}H-diazepam in membrane preparation from cortex was reduced up to the 14th day after birth [14].

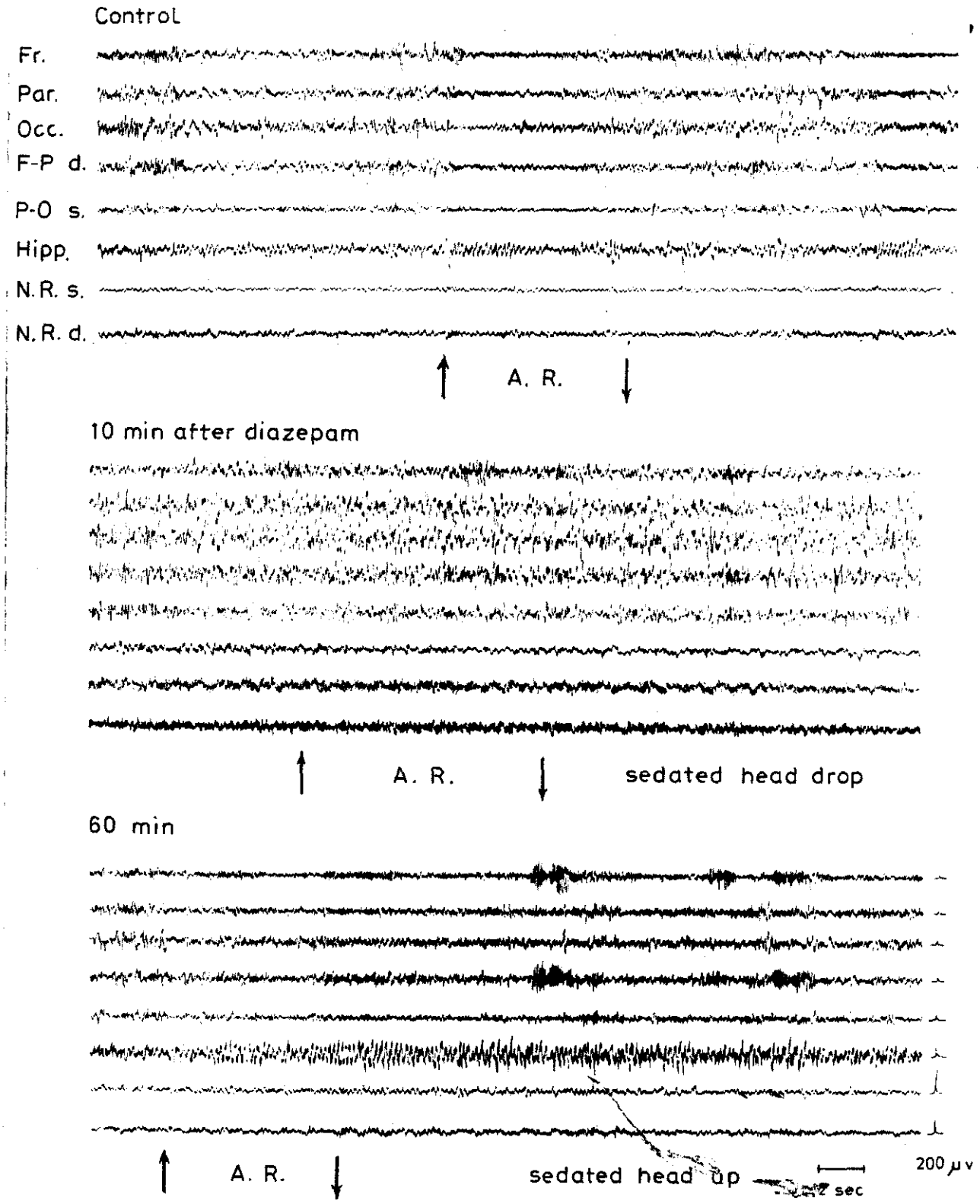

FIG. 1. – EEG effects of acute administration of diazepam (20 mg/kg i.v.) in rabbits. The animals were subjected to the surgical procedure in order to place the electrodes, under local anesthesia, just before the recording. Few seconds after administration of the drug slow waves appear in the cortical leads lasting approximately 10 min; the hippocampus record is disrupted with a decrease of voltage; at the red nucleus level the record shows an increase of voltage and a decrease of frequency. The animal is sedated with head drop and the reaction to external stimuli is absent. Sixty min later at the cortical leads the record shows the presence of trains of spindles and beta-like activity. The hippocampus and red nucleus patterns tend to normal. The animal is sedated and the reaction to external stimuli is present Fr=anterior sensorimotor (frontal) cortex; Par.=posterior sensorimotor (parietal) cortex; Occ.=optic (occipital) cortex; Hipp=hippocampus; N.R. s.=left red nucleus; N.R. d.=right red nucleus.

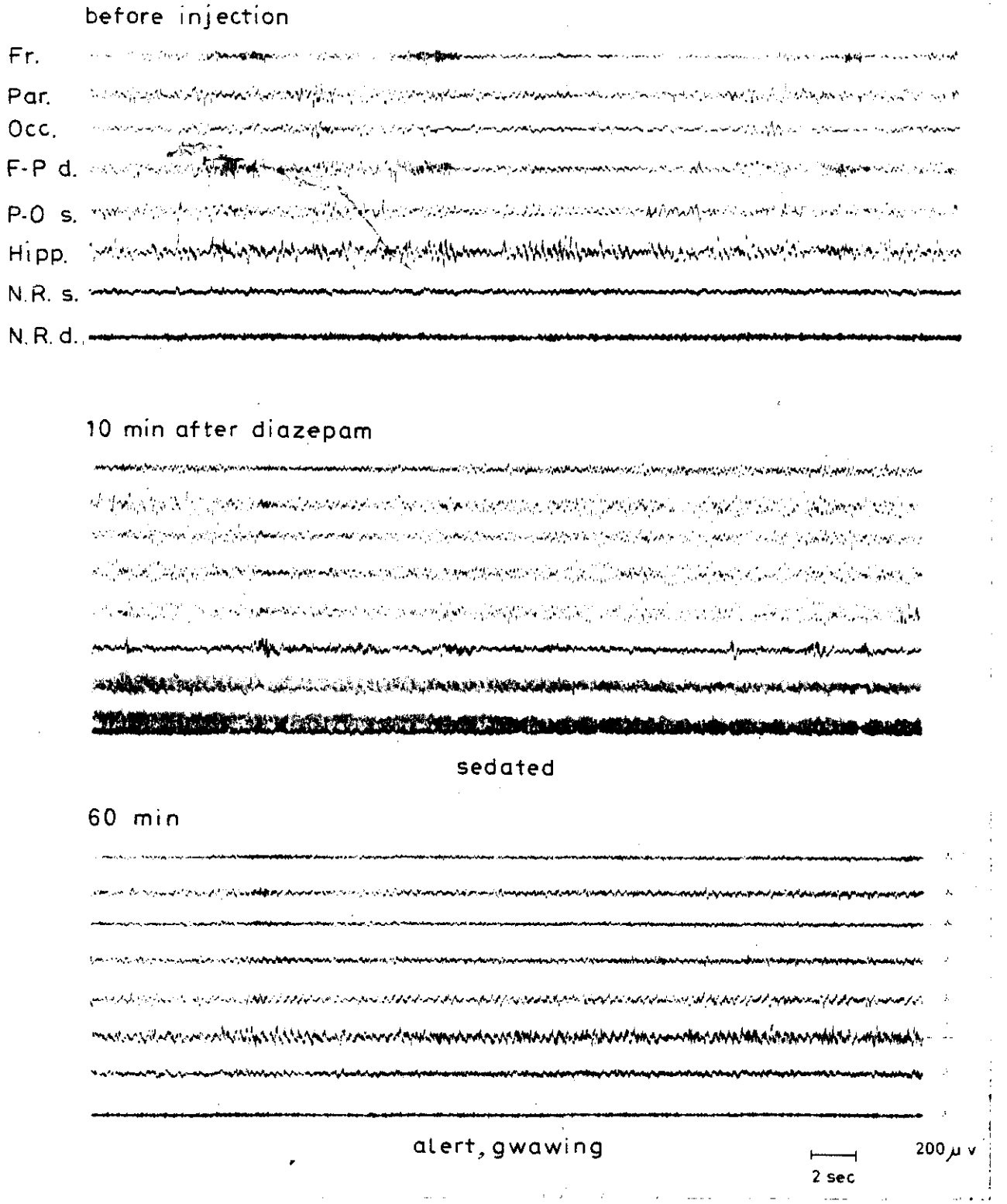

FIG. 2. – EEG effects of chronic administration of diazepam (20 mg/kg i.v.) in rabbits at the 9th day of treatment. The animals were subjected to the surgical procedure in order to place the electrodes under local anesthesia just before the recording. Before the administration of the drug at the cortical leads the record is synchronized; the hippocampal theta waves are disrupted. Few min after injection, 20–30 c/s waves appear in the cortical leads. The hippocampal recording shows a reduction of voltage: at the red nucleus level an increase of voltage and a reduction of frequency are observed. Sixty min after drug administration the EEG evidences the presence of beta-like activity at the cortical level: the hippocampus is still disrupted, while in the red nucleus the record is normal. Fr, Par., Occ., Hipp., NR as described in the Fig. 1.

In the present study the electroencephalographic (EEG) and behavioral effects of chronic administration of diazepam have been considered in view of defining the modifications connected to the development of tolerance.

ELECTROENCEPHALOGRAPHIC STUDIES.

Rabbit. In order to evaluate the EEG correlates of the chronic administration of diazepam, 48 male rabbits received 20 mg/kg i.v. of the drug for 12 days through a cannula chronically implanted in the ear vein. The recordings were carried out in different animals at the 1st, 3rd, 5th, 7th, 9th and 12th day of treatment. On the day of recording under local anaesthesia (2% xylocaine) six screw electrodes were placed on the

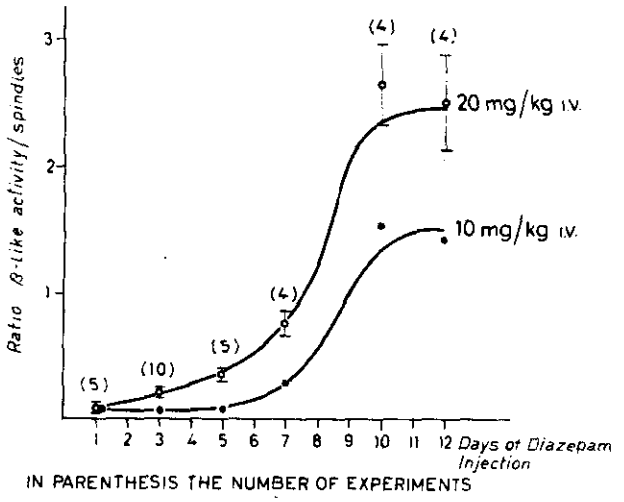

FIG. 3. – Ratio beta-like activity spindles in the cortical EEG throughout the chronic treatment with diazepam (20 mg/kg i.v.) in rabbits. The figure shows the ratio values of the duration of the 20–30 c/s waves (beta-like activity) over the trains of spindles measured within one hour after each administration of diazepam at various days of treatment. The animal were subjected to the surgical procedure in order to place the electrodes, under local anesthesia, just before the recording. The ratio values increase after the 5th day of treatment, reaching the maximal values at the 10th day of treatment. In parenthesis is reported the number of experiments done for each point.

sensorimotor and optic cortices. Concentric deep electrodes were placed at level of dorsal hippocampus and red nucleus. Histological examinations *post-mortem* confirmed the location of deep electrodes. The recording was performed in the restrained non-curarized animals.

On the first day (Fig. 1), the administration of diazepam induced the appearance, within 10 min, of a record rich in slow (2–4 c/sec) high voltage waves in the cortical leads; the hippocampal recording showed a reduction of the voltage with disappearance of the theta rhythm; in the red nucleus, an increase of voltage together with a reduction of frequency was observed. Forty min after diazepam long trains of spindles were present, together with 20–30 c/s waves which resemble those observed in humans after administration of barbiturates (beta-like activity). Conversely, the modifications observed in the hippocampal and red nucleus leads tended to disappeare 40–60 min after the injection.

In the following days of treatment, the administration of diazepam elicited a short-lasting synchronization followed by a longer periods of beta-like activity (Fig. 2). The hippocampal record was disrupted. The red nucleus record showed the same modifications described after the first administration. The arousal EEG reaction to external (vibroacustical) stimuli was absent in the first 10–20 min after drug injection up to the third-fourth day of treatment, while it was present in the following days.

In Fig. 3 are reported the ratios of the occurrence of beta-like activity over the occurrence of the trains of spindles. The values were calculated within one hour after diazepam injection, at various days of treatment. The value increased progressively, reaching the plateau at the 9th day of treatment and lasting up to the 15th day after the end of treatment.

In order to better evaluate the EEG signs of tolerance, starting from the 2nd day after discontinuation of diazepam, observation of the EEG effects after increasing doses of diazepam (0.1–10 mg/kg, i.v.) was carried out.

Doses of 0,7 mg/kg, which in the naive rabbits induced the appearance of slow waves and spindles, in the tolerant animal elicited long periods of beta-like activity (Fig. 4). With higher doses of diazepam (2.5 mg/kg) only the beta-like activity was present in the first hour recording.

Rat. Forty three male albino rats of Wistar strain, chronically implanted with cortical electrodes, were injected i.v. for 10 days with 10 mg/kg of diazepam through a cannula cronically inserted into the jugular vein. The recording was performed in the freely moving animals at the 1st, 3rd, 5th, 7th and 10th day of treatment.

At the first day of treatment slow waves appeared in the record and the EEG reaction to external stimuli was absent. Fifteen-twenty min later, continuous trains of spindles appeared in the record; in the following 20–30 min the trains of spindles alternated with 20–30 c/s waves (beta-like activity) were observed.

In the following days of treatment the period of slow waves, which followed the drug injection, became shorter and an increase of the beta-like activity was observed. After the third-fourth day of treatment, 24 hours after the injection the beta-like activity spread also to the optic cortex and the ratio beta-like activity/spindles, measured within one hour after drug administration increased under treatment, reaching the plateau at the 5th day of injection (Fig. 5). During the withdrawal period the recording evidenced the presence of beta-like activity which disappeared within 3–5 days in the optic cortex and within the 15th day in the sensorimotor cortex.

In order to better evaluate the tolerance signs, from the second day of withdrawal a dose-response curve with diazepam (0.1–10 mg/kg i.v.) was performed. A strong increase in beta-like activity was observed at the dose of 0.7 mg/kg or higher. After the 4th day of withdrawal, this activity tended to disappear from the optic cortex, while in the sensorimotor cortex was present up to the 15th day after the end of treatment.

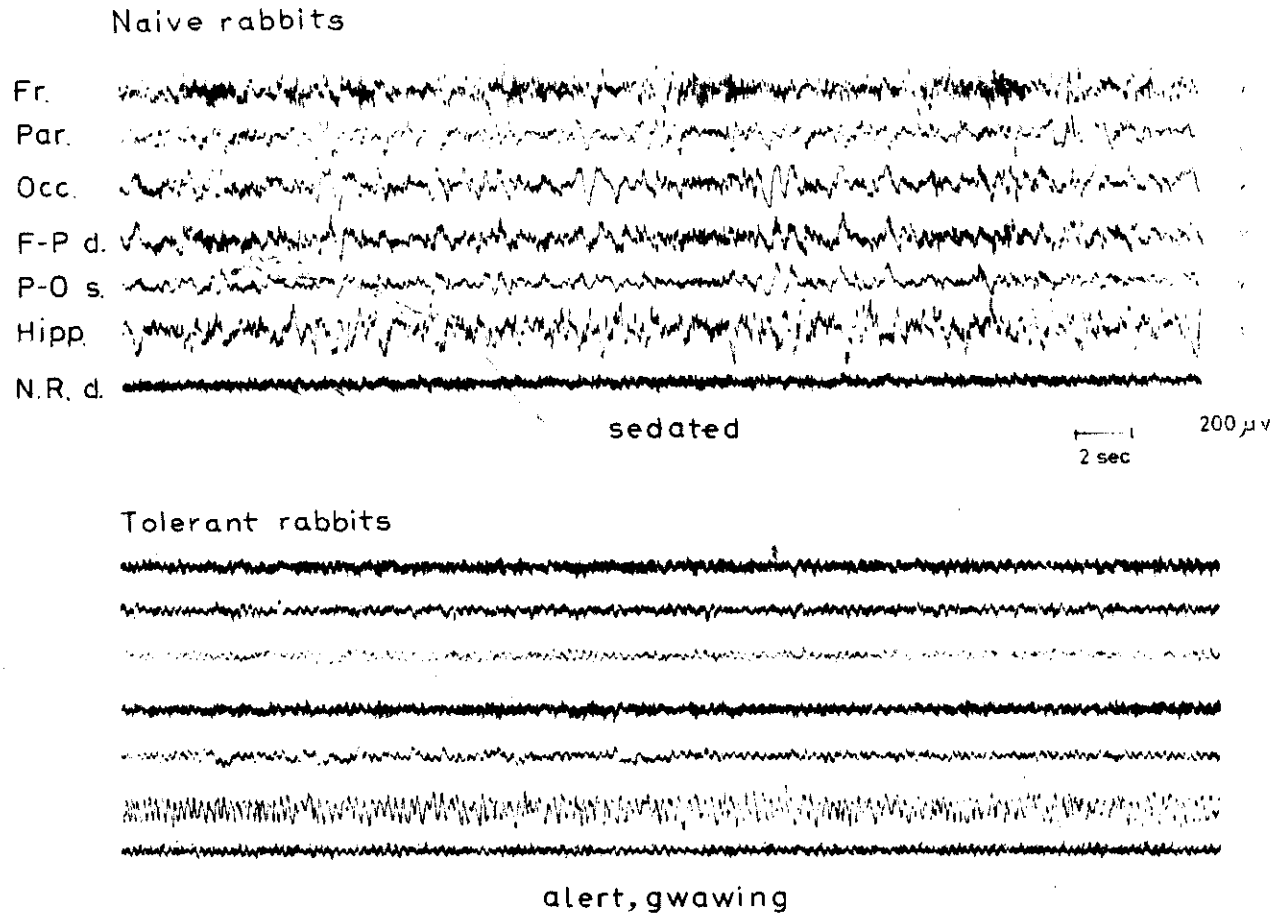

FIG. 4. – EEG effects of acute administration of diazepam (0.7 mg/kg i.v.) in tolerant rabbits. The figure shows the EEG effects observed 10 min after acute administration of diazepam in naive and tolerant rabbits at the 3rd day after the end of treatment with diazepam (20 mg/kg i.v. for 12 days). In the cortical leads only beta–like activity is present in tolerant rats; the hippocampal pattern is more activated, while no significant differences can be observed between the two animals in the red nucleus pattern. The animals were subjected to the surgical procedure in order to place the electrodes, under local anesthesia, just before the recordig. Leads as reported in theFig. 1

BEHAVIORAL STUDIES.

It has been reported that a short–lasting phase of stimulation is present after administration of both diazepam and GABAergic drugs in rats and rabbits [15]. In our experimental conditions the daily administration of diazepam induced in both rats and rabbits in the first day of treatment an immediate sedation accompanied by myorelaxation and impairment of the righting reflex. Starting from the 2nd–3rd day of treatment sniffing, hyperirritability, twitching of ears, slight motor excitation, compulsive gnawing and, in 70 % of the animals, compulsive eating appeared. Upon treatment and during the withdrawal period no significant changes of water and food consumptions were observed nor a significant modification of body weight.

EFFECTS OF NALOXONE IN TOLERANT RATS.

It is now generally accepted that the benzodiazepine derivatives exert their pharmacological effects by potentiating the GABA synaptic transmission. In addition, it has been recently demonstrated that opiates can affects several pharmacological actions of benzodiazepines by interfering with the transynaptic phenomena of the GABA transmission (see for ref. 3). It seemed therefore appropriate study the EEG effects of opiate agonist and antagonist in rats during the withdrawal period after administration of diazepam.

Morphine and naloxone, at doses ranging from 0.5 to 10 mg/kg i.v. did not affect significantly the EEG modifications observed during the withdrawal period. However, naloxone (5 mg/kg i.v.), but not morphine, reversed the EEG and behavioral effects induced by administration of diazepam (0.7 mg/kg i.p.) in tolerant rats. As shown in fig. 6, the ratio between the occurrence of beta–like activity and of spindles, measured within one hour after administration of 0.7 mg/kg i.v. of diazepam, was 0.53 in naive rats, but reached the value of 2.12 and 2.41 in tolerant rats at the 2nd and 4th day of withdrawal period, respectively. In these experimental conditions, the administration of naloxone strongly reduced the occurrence of the beta–like activity and the ratio decreased to 0.31 (Fig. 6). In addition, the behavioral stimulatory effect was blocked.

CONCLUSIONS.

Controversial data exist in the literature on the onset of tolerance to the various effects of diazepam. The present experiments have demonstrated that tolerance

to the sedative effects of the drug has also an EEG counterpart, consisting of the appearance of beta-like activity both in rabbits and rats. This activity seems also characteristic of the withdrawal period observed upon discontinuation of the drug. On the other hand no evidence was obtained of tolerance to the myorelaxant effects of diazepam. This was accompained by the lack of modifications in the red nucleus pattern, i.e., increase of voltage and decrease of frequency, induced by administration of diazepam. There are data in the literature indicating that the rubro–olivo–cerebellar–rubral loop is involved in the maintenance of muscle tonus [16].

This would indicate that there are central effects of diazepam which are less sensitive of others to the development of tolerance.

It is difficult at present to explain the effect of naloxone found in tolerant rats. This drug is an opiate antagonist but at high doses also exhibits a GABA antagonistic effect [17].

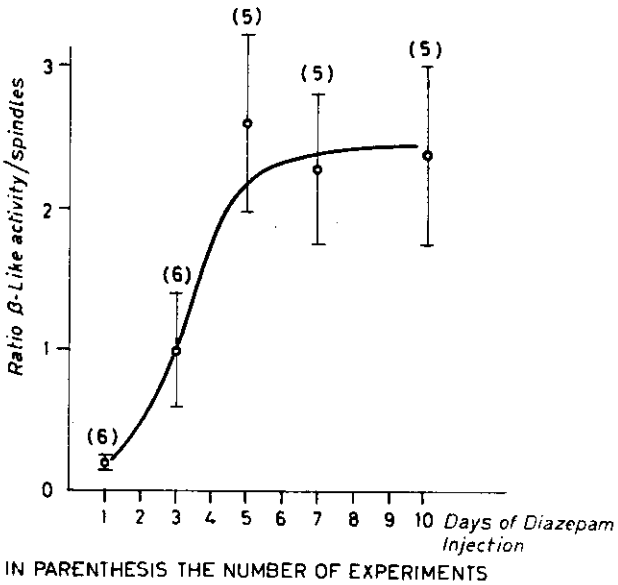

FIG. 5. – Ratio beta–like activity/spindles in the cortical EEG throughout the chronic treatment with diazepam (10 mg/kg i.v.) in rats. The figure shows the ratio values of the duration of the 20–30 c/s waves (beta–like activity) over the time of duration of the trains of spindles, measured within one hour after each administration of diazepam, at the various days of treatment. The ratio values increase immediately after the first day of treatment reaching the plateau at the 5th day of treatment. In parenthesis are reported the number of experiments done for each point.

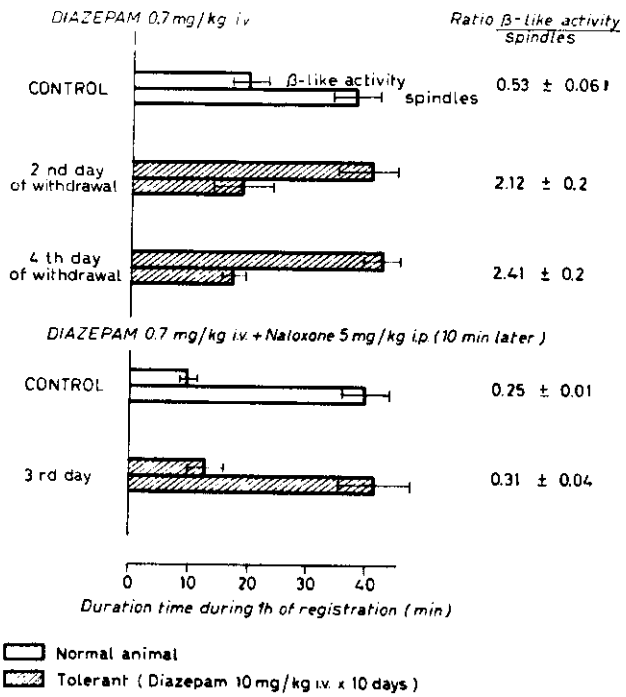

FIG. 6. – Naloxone blockade of the beta–like activity induced by acute administration of diazepam (0.7 mg/kg i.v.) in tolerant rats. The duration of the occurrence of 20–30 c/s waves (beta–like activity) and spindles were measured within one hour after drug administration in 27 rats. In the upper part, the histograms show the occurrence of the two cortical patterns in the EEG after acute diazepam in normal animals (control) and in tolerant rats at the 2nd and 4th day after the end of treatment with 10 mg/kg i.v. of the drug. In tolerant rats the beta–like activity overcome the occurrence of the trains of spindles, characteristics of administration of benzodiazepines. In the lower part, the histograms show the effects of administration of naloxone (5 mg/kg i.p.) on the cortical EEG modification induced by acute administration of diazepam (0.7 mg/kg i.v.) in tolerant rats. At the third day of the withdrawal period the occurrence of beta–like activity after acute administration of the diazepam is counteracted by administration of naloxone. For each group the upper column indicates the time of occurrence of beta–like activity, while the lower one indicates the time of occurrence of trains of spindles.

Its capability in reverting at high doses the EEG and behavioral effects of diazepam tolerance, as shown during the withdrawal period, lets us hypothesize that the tolerance to diazepam might be also linked to an increased activity of the GABA transmission. However, the possibility cannot be excluded that an increase of the opiate modulatory activity on GABA synaptic transmission might be relevant in establishing the tolerance to benzodiazepines.

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Poster session

Spectra of psychotropic activity and neurochemical mechanisms of action of bi- and tetracyclic antidepressants

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Summary. – *The antidepressive effect of new drugs was studied and compared to classic tricyclic antidepressants using model behavioral depressions on cats and rats.*

Riassunto. – *E stato studiato l'effetto antidepressivo di nuovi farmaci in confronto con antidepressivi triciclici noti, usando modelli sperimentali volti a riprodurre la depressione comportamentale nei gatti e ratti.*

Introduction.

Despite certain achievements in the field of pharmacotherapy of depression, design and study of new compounds are still in progress. Search for new antidepressants has revealed a number of active agents (nomifensine, trazodone, befuraline, pyrazidol, etc.) structurally different from the classic tricyclic antidepressants. These new agents proved also to have distinct pharmacological and neurochemical properties.

In this paper the results are reported of a study of antidepressants of bi– and tetracylic structure, tricyclic compounds and monoaminoxidase (MAO) inhibitors, carried out by comparing the spectra of their behavioral and neurochemical action.

Methods.

In chronic experiments on 22 adult male cats (weighing 3,8–4,2 kg) the effect of befuraline and pyrazidol on the behavioral changes induced by reserpine (18–20 hours in advance 0,1 mg/kg s.c.) was studied. The animals were treated twice a day with 45 mg/kg of befuraline or 15 mg/kg of pyrazidol injected i.p. In other experiments reserpine (the same dose) was injected 2 hours after treatment with antidepressants: befuraline 45 mg/kg, pyrazidol 15 mg/kg, nialamide 15 mg/kg, chlorimipramine 5 mg/kg.

Behavioral « emotional » reactivity, somatomotor and neurovegetative manifestations were estimated. The zoosocial interaction including the evaluation of sociability, conflictness, dominance, hostility, competitiveness was also examined. A quantitative method using a 5–point scale was applied to the evaluation of behavioral reactions [1]. Significance of results was evaluated with the Student's t–test.

The action of the drugs on the « open field » test was studied using 100 albino (mongrel) mice weighing 18–20 g. The animals were put for 3 minutes in a box 50 cm diameter, height of the walls 15 cm, floor divided into 28 equal squares with holes, with a 150 watt lamp placed 60 cm above the surface. The drugs were injected intraperitoneally in doses of 25 mg/kg (0.1 ml of aqueous solution per 10 g of body weight). The parameters followed were: horizontal activity (number of crossed squares) (HA), vertical activity (number of rearings) (VA), peeping into the holes (PH), grooming (GR). The estimation was based on the data of three independent observators.

Other properties evaluated were: the ability to potentiate amphetamine stereotypies (amphetamine 3.5 mg/kg was injected i.p. 30 min after administration of the drugs), the « head twitching » induced by 5–hydroxytryptophan (75 mg/kg i.p., 45 min after the drugs) and the picrotoxin convulsions (picrotoxin 2.5 mg/kg i.p., 45 minutes after the drugs), as well as the antagonism of the effects of tetrabenazine (blepharoptosis, hypotermia) administered subcutaneously (40 mg/ kg) 30 min after befuraline.

The effect of the drugs on the neurotransmitters uptake system was studied on crude synaptosomal preparation prepared by centrifugation of 10 % rat brain homogenate in 0.32 M saccharose at 1000 g within 10 min, followed by centrifugation of the supernatant at 11000 g for 20 min. The sediments containing synaptosomes, mitochondria, and myelin were resuspended in 0.7 ml of 0.32 M saccharose per 1 g of the initial mass of the brain. 50 µl of the prepared suspension of crude synaptosomal preparation (1 mg of protein, on the average) were added to 1 ml of incubation medium containing in mM: 100 NaCl, 6 KCl, 2 $CaCl_2$, 1.14 $MgCl_2$, 5 Na_2HPO_4, 10 glucose, 100 saccharose, 0.125 pargiline and 30 tris–HCl buffer pH = 7.4, labeled transmitter and pharmacological drugs in corresponding concentrations. The concentrations of transmitters used were: for ^{3}H–serotonin 83 nM, specific radioactivity 12 Ki/mmole « Amersham », for GABA–^{3}H–10

MM with specific radioactivity 10 Ki/mmole « New England Nuclear ». The incubation was conducted at 37°C for 20 min with continuous shaking. Transmitter binding was stopped by cooling up to 0–5 °C. Synaptosomes were isolated from the incubation medium and the activity of bound transmitters was recorded according to a modified technique of Snyder and Coyle [2]. Radioactivity was measured by « Intertechnique » scintillation counter, calculating the mean number of disintegrations per minute. Protein was determined according to Lowry. The data was statistically treated calculating the mean values and their confidence limits at P = 0.05.

RESULTS.

The symptoms observed in the reserpinized animals consisted of the following: ataxia, catalepsy, decreased spontaneous locomotor activity, decrease up to a complete disappearance of motivated activity, changes in emotional reactivity (suppression of reactions to positive stimuli and enhanced passive-defensive reactions to negative stimuli), neurovegetative disturbances (myosis, relaxation of nictitating membrane, hypotermia). The peak of depression was observed on day 2–3; return to normal took 6–7 days.

The antidepressants studied had different effects on the dynamics of reserpine-induced depression. Befuraline completely prevented the development of behavioral manifestations of the reserpine effect. The recovery of drive activity and purposeful behavior of the animal was the important step in the development of the antidepressant effect (Fig. 1). In experiments on mice befuraline increased the activity of central noradrenergic and serotoninergic systems (Tab. 1), but it had no significant influence on the activity of dopaminergic systems, while potentiating amphetamine hyperactivity, decreasing tetrabenazine hypotermia and ptosis, potentiating hyperkinesia induced by 5-hydroxy-

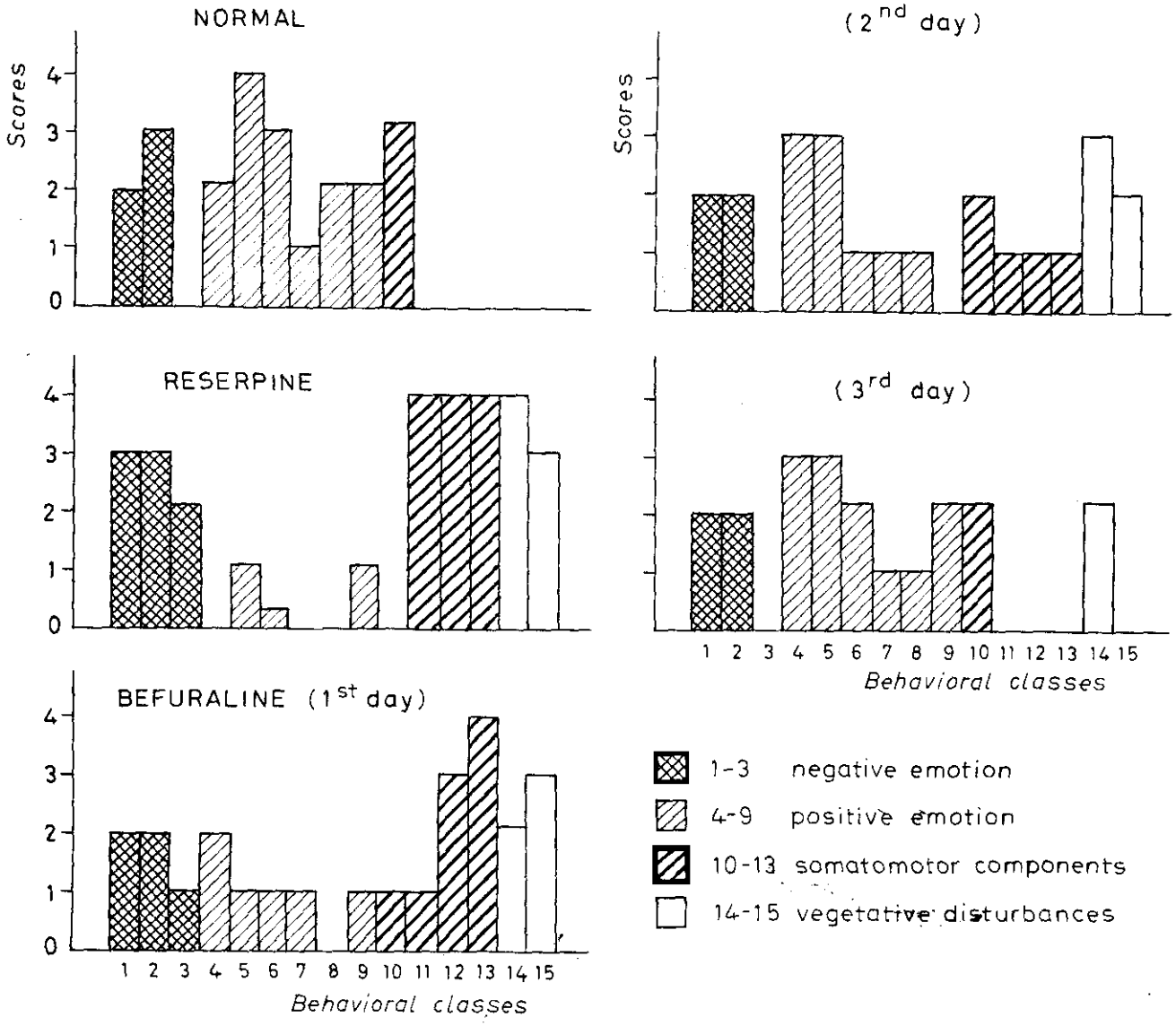

FIG. 1. – Effect of befuraline on reserpine-induced depression in cats. *Components of the negative emotional state*: 1) active-defensive reactions; 2) passive-defensive reactions; 3) negativism. *Components of the positive emotional state*: 4) curiosity; 5) food behavior; 6) contentment; 7) play; 8) hunting; 9) arousal reaction. *Somatomotor components*: 10) spontaneous locomotor activity; 11) non-expressiveness of the emotional reactions; 12) depressive posture; 13) catalepsy. *Vegetative disturbances*: 14) myosis; 15) relaxation of the nictitating membrane.

Table 1. – *The screening activity of antidepressants and their influence on the « open field » behavior of mice.*

DRUG	Potentiating effects of			Antagonism with tetrabenazine	Open field			
	Picrotoxin ED_{50}	5 – HTP (mg/kg)	amphetamine		HA	VA	PH	GR
DMI	25,0 (21–28)		11,2 (7,8–13)	+ —	↑	↑	↑	↓
Befuraline	58,0 (47–70)	82,0 (71–89)	—	+ —	↑□	↓	↓	↓
Trazodone	—	7,22 (6,8–8,3)		+ —				
Pyrazidol	20,1 (17–22)	9,82 (7,8–11,0)	20,0 (18–23)	+ —				

tryptophan and picrotoxin convulsions. The duration of the amphetamine stereotypies did not change. The activity of the animals in the open field was slightly enhanced. According to Boksay et al. [3], the effect of befuraline is due to activation of the central adrenergic structures which is in conformity with our findings. No effect of befuraline on dopamine uptake by brain synaptosomes was found.

For pyrazidol a reversible inhibition of MAO A and an inhibition of noradrenaline uptake has been reported [4]. Also this drug prevented the development of reserpine-induced behavioral depression. The antidepressant effect of pyrazidol consisted of a recovery of the reactions to positive test-stimuli and of a decrease of the negative emotional state. Neurochemical data indicate that pyrazidol is more active than the other drugs in potentiating picrotoxin convulsions and is only slightly different from trazodone in its effect on serotoninergic mechanisms. Pyrazidol potentiates amphetamine sterotypies, decreases tetrabenazine ptosis and hypotermia (Tab. 1). These results are in agreement with the findings of Mashkovsky and Andreyeva [5] and suggest the involvement of the serotoninergic system in the antidepressant action as the enhancement of the spectrum of positive emotions.

Neither befuraline nor pyrazidol influenced the somatomotor and the vegetative components of the behavioral depression due to reserpine. Chlorimipramine, a serotonin uptake inhibitor [6], was somewhat less potent in preventing the development of behavioral depression manifestations and did not eliminate vegetative disturbances. In low concentration, in which the specific effect of the drugs develops, only desmethylimipramine (DMI), like trazodone, produced a marked potentiation of ^{3}H-serotonin uptake (67–79 % inhibition) and slighly affected the uptake of ^{3}H-GABA (Tab. 2). In higher concentration (500 μ M) it inhibited ^{3}H-GABA reuptake like the other drugs. It is evident that when antidepressants are used in high doses their individual differences are not observed in either behavioral or neurochemical study. The inhibition of ^{3}H-GABA uptake after high doses of the drugs is related to their non-specific membrane-tropic action.

Table 2. – *The influence of antidepressants on the ^{3}H-GABA and ^{3}H-serotonin uptake by crude synaptosomal preparation from rat brain.*

DRUG	% Neurotransmitter's uptake			
	50 mkM		500 mkM	
	Serotonin	GABA	Serotonin	GABA
Control	100±9	100±9	100±10	100±10
DMI	21±3	77±9	19±2	12±2
Befuraline	86±10	121±15	30±4	25±5
Trazodone	33±4	96±10	22±3	91±10
Pyrazidol	71±8	99±15	26±3	31±4

Footnote: 10 nM of ^{3}H-serotonin and 3.5 μM of ^{3}H-GABA per 1 mg of protein crude synoptosomal fraction within 20 min at 37 °C were considered as 100 %.

The prolonged reserpine effects on the central and peripheral monoaminergic neurons have been attributed to the irreversible binding of small quantities of the alkaloid in specific sites of the granular membrane and to the inhibition of ATP and Mg^{++} -dependent uptake in the granules [6, 7]. Small quantities of reserpine were found in the brain of rats and mice up to 5 days after treatment [8, 9]. Probably there is a balance between lipid (large nonspecific) and granular (small specific) pools of reserpine, the lipid pool may be the source of reserpine for prolonged inhibition of the granular uptake leading to the depletion of monoaminergic systems [10]. Dahlstrom and Haggendal [11] found out that noradrenaline recovery in peripheral nerves developed only on the 7th day after single reserpine injection.

The imipramine-like drugs do not modify the initial concentrations of reserpine in the rat brain [12]. It is possible that the antidepressants prevent irreversible binding of small quantities of reserpine in receptor sites of the brain, moreover that there was no correlation between monoamine content and reserpine binding in the brain [13]. This correlation was found in the peripheral terminals in accordance with the density of noradrenergic innervation [14].

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Thyrotropin content in serum of rats motivated and non-motivated to alcohol

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Summary. – *It has been shown that alcohol-motivated Wistar female rats after 10 days of repeated ethanol administration have a decreased cold-or TRH-stimulated TSH level in the blood serum under physical dependence and abstinence. There were no changes of TSH secretion in animals non-motivated to alcohol. Acetaldehyde inhibited significantly the TSH cold-response but did not influence TRH-induced TSH secretion.*

It is suggested that repeated ethanol administration causes a hypofunction of both hypothalamic TRH neurons and anterior pituitary thyrotropic cells. Partially it may depend on acetaldebide inhibitory action on hypothalamic level of regulation of thyroid gland functioning.

Riassunto. – *Ratti Wistar dipendenti dall'alcool hanno bassi livelli di TSH in risposta alla stimolazione con TRH o dopo esposizione al freddo. L'acetaldeide inibisce la risposta al freddo ma non influenza quella al TRH.*

Introduction.

Studies on motivation and non-motivation to ethanol are one of the main aspects of the research of drugs for the prevention and treatment of alcoholism. It was previously reported that ethanol causes aggression in non-motivated animals and has a calming effect in motivated animals [1]. Ethanol activates positive reinforcement in motivated animals but does not work in the animals which reject ethanol [2]. The rats motivated to ethanol have been found to have an increased activity in the hypothalamic tyrosine hydroxylase [3], an increased 5-HT content in some brain structures [4] and an increased activity of ethanol metabolizing enzymes [5].

There are some data about the difference in the effects of ethanol on the hypothalamus-hypophysis neurosecretory system in the animals which prefer or reject ethanol [6]. These effects seem to depend on the duration of the treatment and on the severity of the ethanol intoxication [7-9].

The present work was devoted to study the effect of repeated ethanol administration on the thyrotropin concentration in serum of rats either motivated or non-motivated to ethanol. Various stimulation tests were performed both during the state of physical dependence and during the state of abstinence.

Material and Methods.

Animals. 156 female Wistar rats, weighing 150-220 g were used. The rats were kept 3-5 in each cage in a room artificially illuminated from 7 a.m. to 7 p.m. They were given standard laboratory pellets (iodine content 0.5-1 mg/kg) and tap water *ad libitum*.

Experimental designs.

I) *TSH secretion during ethanol dependence or abstinence.* Previous studies [5] have revealed a reverse correlation between the sleeping-time (induced by a narcotic dose of 25 % ethanol, 4.5 ml/kg i.p.) and the volume of the ethanol consumed when the animals had a free choice between ethanol (15 %) and water. In our experiments, the rats with sleeping-times of 60 min or less were classified as short-sleepers (SS) or motivated to ethanol and those sleeping 120 min or more as long-sleepers (LS) or non-motivated to ethanol. LS rats consume only 0-3 ml of 15 % ethanol per day under a free-choice situation, whereas SS rats consume 10-40 ml per day [5]. One week after the sleeping test, the chronic treatment was started according to the modification of the method used by Gothoni and Ahtee [10]. In brief, 10 % (V/V) ethanol in water was given 3 times daily (every 8 hours) by a gastric tube in increasing volumes: on the 1st-3rd day 8 ml/kg, on the 4th-6th day 15 ml/kg and on the 7th-10th day 20 ml/kg. Control animals were given equal volumes of water. The experiments were performed on the 10th day either 4-6 h after the last ethanol dose (physical dependence) or 16-18 h after the last dose (abstinence). The following tests were performed. 1) Basal serum TSH level were measured. 2) The TRH-induced TSH secretion was studied by giving 50 ng/100 g of body weight of TRH i.p. and killing the rats 30 min later. Serum TSH levels were measured. This test monitors the function of the anterior pituitary thyrotrophic cells. 3) The cold-induced (the rats kept at a temperature of + 4 °C for 30 min) TSH secretion

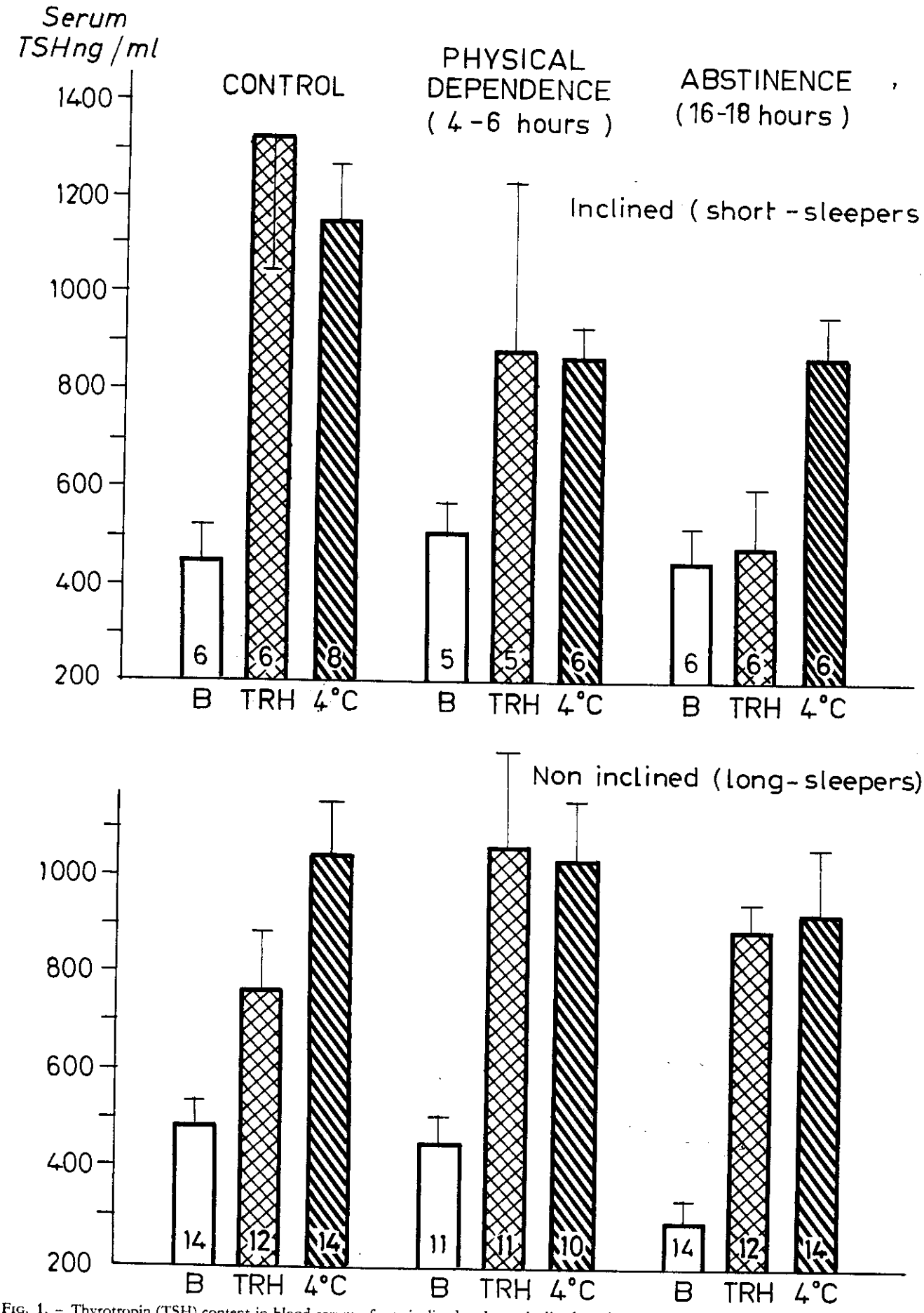

FIG. 1. – Thyrotropin (TSH) content in blood serum of rats inclined and non-inclined to alcohol in the state of physical dependence and abstinence. Ordinates: serum content in ng/ml. Abscissa: Basal (B), TRH-stimulated (TRH) and cold-induced (4 °C) TSH levels. Figures in the columns indicate the number of experiments.

is a measure of the activity of the hypothalamic TRH neurons.

II) *Effect of acetaldehyde on TSH secretion.* The rats (both SS and LS) were given either 0.9 % saline or 100, 200 or 300 mg/kg of acetaldehyde i.p. Then the rats were transferred to + 4 °C and killed by decapitation after 30 min. The blood of the whole trunk was collected and serum TSH assayed. Some rats were left at a room temperature. In another study, the rats were given either 0.9 % saline or 200, 300 or 450 mg/kg of acetaldehyde i.p. together with 50 ng/100 g or 150 ng/100 g of TRH at the same time. After 30 min the rats were decapitated and serum TSH assayed.

III) *TSH radioimmunoassay.* Serum TSH was measured using a radioimmunoassay kit which was a gift from NIAMDD, the Rat Pituitary Program, Bethesda, Maryland. It contained TSH for radioiodination, antithyrotropin antiserum and a rat TSH standard (biol. potency 0.22 USP bovine units/mg in the McKenzie assay). The results are expressed in ng/ml of this standard (TSH-RP 1).

IV) *Statistics.* Arithmetic means, SEMs and SDs were calculated. Student's t-test was used for comparison of two means. In case of three or more means an one-way analysis of variance was first applied.

Results.

1) *Behaviour.* After 5 days on ethanol, the rats had behavioural changes which resembled withdrawal syndrome (stiffness and tremor of the body and tail, moderate rigidity, aggression). These symptoms were most pronounced 16–18 h after the last ethanol dose. Aggression was the most typical sign.

2) *TSH secretion during physical dependence and abstinence.* Without any ethanol treatment, the ethanol-motivated rats had a greater TRH-response than the non-motivated rats, but there was no difference in the basal or cold-stimulated TSH levels. Under physical dependence, in the motivated (SS) rats both the TRH- and cold-induced TSH secretions were somewhat decreased. This was not seen in the non-motivated (LS) rats. In the state of abstinence, the motivated rats did not have any TRH response at all and also the response to cold exposure was significantly inhibited. Again, the non-motivated rats had normal responses (Fig. 1).

Table 1. – *The effect of acetaldehyde on the cold-induced TSH secretion.*

TREATMENT	Serum TSH (ng/ml)	n	P
0.9 % saline, + 30 °C	920±72	4	0.001
0.9 % saline, + 4 °C	3,085±579	6	—
Acetaldehyde 100 mg/kg + 4 °C	3,156±397	6	NS
Acetaldehyde 200 mg/kg + 4 °C	1,526±139	7	0.05
Acetaldehyde 300 mg/kg + 4 °C	1,310±313	6	0.05

Mean ± SEM. n = number of animals.

3) *Effect of acetaldehyde on TSH levels.* Acetaldehyde (200 and 300 mg/kg) inhibited significantly the TSH cold-response (Tab. 1), but only 450 mg/kg inhibited the TRH-induced TSH secretion (Tab. 2).

Discussion.

The data obtained suggest that the ethanol-motivated rats (SS) have a normal hypothalamus-anterior pituitary axis. However, repeated ethanol administration seems to cause a slight hypofunction of both the hypothalamic TRH neurons and the anterior pituitary thyrotrophic cells. In the state of abstinence TRH-response is absent suggesting an impairment of the anterior pituitary gland functions. This may be related to an increased dopaminergic activity during abstinence [11, 12], because dopaminergic drugs have been shown to inhibit TSH secretion, although not at the anterior pituitary level [13]. In the ethanol non-motivated rats (LS) only basal TSH levels were significantly decreased in the state of abstinence, but both the TRH- and cold-stimulation tests gave normal results. A partial inhibition of the cold-response and especially the inhibition of the TRH-response by high doses of acetaldehyde are evidently unspecific and related to the effect of various anaesthetics as was shown by Mannisto et al. [14].

Table 2. – *The effect of acetaldehyde on the TRH-induced TSH secretion.*

DOSE OF ACETALDEHYDE	Dose of TRH 0	Dose of TRH 150 ng/100g	Dose of TRH 50 ng/100g
		Serum TSH, ng/ml	
0	519±126 (6)	1,423±373 (6)	1,622±211 (6)
200 mg/kg	—	1,832±303 (7)	2,043±529 (7)
300 mg/kg	—	1,997±470 (7)	2,501±354 (7)
450 mg/kg (**)	—	842±182*(4)	495;2,438 (2)

Mean ± SEM. Number of animals in parenthesis. (**) LD_{50} of acetaldehyde. (*) $P < 0.05$ vs. the corresponding TRH-saline control.

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Pharmacological and electrophysiological differences between γ-hydroxybutyric acid and GABA-mimetic drugs

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Summary. – *In this paper are presented pharmacological and neurophysiological data supporting the concept that GHBA is not a GABA-mimetic drug and that its actions are similar to those of succinic semi-aldehyde.*

Riassunto. – *Sono presentati dati farmacologici e neurofisiologici che dimostrano che l'acido γ-idrossibutirrico non è un gaba-mimetico, ma piuttosto ricalca gli effetti dell'aldeide emisuccinica.*

Introduction.

It is widely accepted that γ-hydroxybutyric acid (GHBA) may be considered a GABA-mimetic agent [1–4]. Moreover it has been suggested that GABA is metabolized into GHBA and viceversa [5, 6]. Controversial reports are available on the pharmacological actions of GHBA and on its possible modifications of the brain GABA content [7–9]. In this paper are presented pharmacological and neurophysiological data supporting the concept that GHBA is not a GABA-mimetic drug and that its actions are similar to those of succinic semi-aldehyde (SSA).

Pharmacological studies.

A) *The antagonism of bicuculline and of AOAA induced convulsions.*

Bicuculline is a GABA-antagonist and may cause convulsions when administered in sufficiently high doses. GABA-mimetic agents antagonize this effect. Table 1 shows that GHBA does not antagonize this type of convulsions, while other GABA-mimetics drugs are effective. Another difference between GHBA and GABA-mimetic agents was evidentiated using the antagonism to convulsions induced by amino oxyacetic acid (AOAA).

Table 2 shows that this type of convulsions are antagonized by SSA, by valproic acid and by GHBA, but not by GABA cetyl-ester, a GABA-mimetic molecule originally described in our laboratory [10].

B) *GHBA and hypoxic conditions.*

It has been described that GHBA may increase the survival time in animals exposed to hypoxic conditions [11, 12]. In Table 3 the effects of GHBA, SSA, valproic acid and AOAA in two different models of hypoxia are reported.

The results suggest that GHBA, SSA and valproic acid have a profile of action completely different from AOAA. At the dose used, AOAA increased brain GABA content by 270 %. These data suggest that the increase of the survival rate and of the survival time caused by SSA, GHBA and valproic acid [13–14] in hypoxic conditions is not due to their effects on brain GABA levels.

Electrophysiological studies.

C) *The comparison of the effect of GABA-mimetic drugs and GHBA on electrophysiological indices of cortical inhibition.*

The recovery cycle of the evoked potentials is known to be one of the most informative indices of the degree of inhibitory processes. Using this test, we have differentiated the effects of GABA-mimetic drugs and GHBA. The recovery cycle of the primary cortical response evoked by the stimulation of the tooth pulp in the rabbit is characterized by a non-constant phase of postexcitatory facilitation which is most marked when a 5 msec interval between conditioning and testing impulses is followed by the phase of postexcitatory depression lasting up to 30 msec. CE-GABA in doses of 5–10 mg/kg caused a marked increase in postexcitatory depression (Fig. 1A, B). In the recovery cycle of corticocortical response recorded in the motor cortex during the stimulation of the sensory area which normally has a more marked and longer phase of postexcitatory facilitation (20–100 msec) CE-GABA completely eliminated the facilitation phase. A similar effect was produced by intraventricular GABA as well as by the earlier studied benzodiazepine derivatives (clonazepam, lorazepam, diazepam, chlordiazepoxide) [14, 15]. These effects were competitively antagonized by bicuculline. The actions of GHBH

are different and dependent upon the dose. When 30–500 mg/kg were administered, GHBA decreased the threshold of the evoked potentials and increased the degree of the facilitation to the testing response. At high doses (1500–2000 mg/kg) it increased the threshold of the conditioning responses and somewhat increased postexcitatory depression of the testing primary response (Fig. 1B, C, D). These changes of excitability are associated to EEG modifications. Such nonselective potentiation of the inhibitory processes coupled with the reduction of the excitatory ones makes the effects of high doses of GHBA similar to that of barbiturates. Thus, in a wide dose range, GHBA was not characterized by the ability to imitate the effect of GABA-ergic compounds on the inhibitory processes but by the ability to decrease GABA-ergic inhibitor. It can be supposed that the ability of GHBA to cause characteristic spike-like discharges

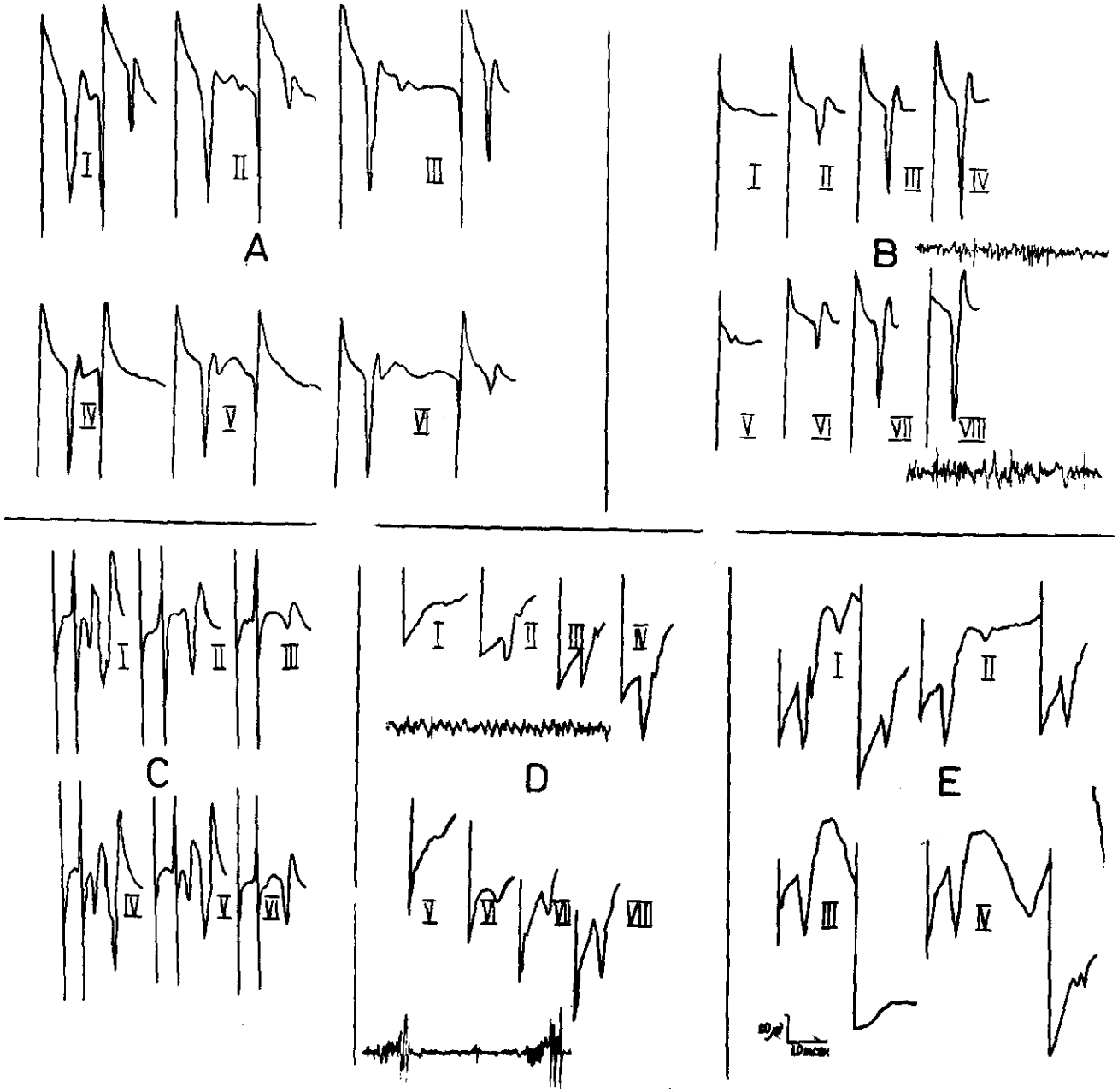

FIG. 1. – Effect of CE-GABA (A, B) and GHBA-Na (C, D, E) on the recovery cycle of cortical potentials evoked by tooth pulp stimulation. A) Influence of CE-GABA on the postexcitatory depression phase. The interval between conditiong and testing potentials is 15 (I, IV), 20 (II, V) and 30 (III, VI) msec. I, II, III: control; IV, V, VI: 30 min after CE-GABA 6 mg/kg intravenously. – B) Influence of CE-GABA on the threshold current intensity at which potentials appeared. Upper row: control- I-3 mA, II-4 mA, III-6 mA, IV-8 mA; Lower row: 30 min after CE-GABA V-4 mA, VI-6 mA, VII-8 mA, VIII-10 mA. – C) Effects of GHBA on the postexcitatory facilitation. The interval between conditioning and testing stimuli is 5 msec; upper row: control, lowet row: 60 min after GHBA 50 mg/kg i.v. I, IV-8m A, II, V-6 mA, III, VI-4 mA. – D) Effect of a high GHBA dose (1500 mg/kg) on the threshold of the appearance of potentials. Upper row: control -I, II, III, IV – 10, 12, 20, 23 mA respectively, lower row: 30 min after GHBA: V, VI, VII, VIII-25, 30, 40, 50 mA respectively. – E) Deepening of postexcitatory depression by GHBA in high doses. The interval between conditioning and testing responses is 20 msec (I, III) and 30 msec (II, IV). Upper row: control (threshold is 20 mA); lower row: 30 min after GHBA 1500 mg/kg (threshold 50 mA). Calibrations: 10 microvolts, 10 millisec. Each potentials is the result of averaging (by multichannel analyzer) of 30 subsequent responses. Experiments carried out in non anesthetized curarized rabbits.

Table 1. – *Antagonism to bicuculline-induced convulsions.*

	Control	Muscimol (05.mg/kg)	GABA CE (10mg/kg)	AOAA (25mg/kg)	GHBA (1000mg/kg)
% Convulsing animals	98	50	50	500	100
Degree of convulsions	60–80	22–80	20–80	28–80	75–80
Death rate (%)	40	10	5	15	20

Bicuculline (3 mg/kg i.p.) was administered 180 min after AOAA and 30 min after muscimol, GABA CE or GHBA. The degree of the convulsions was evaluated on arbitrary basis (80 = max). 20 animals for each group were used.

Table 2. – *Antagonism to AOAA-induced convulsions.*

	Control	SSA (500mg/kg)	GHBA (500mg/kg)	Valproic ac. (300mg/kg)	CE-GABA (10mg/kg)
% Convulsing animals	95	10	25	15	95
% Mortality	30	0	0	0	20
Latency (min)	11±0.2	40±2	35±1.7	37±1.5	12±2

AOAA (150 mg/kg) was injected i.p. Twenty rats for each group were used. Values are percentage or mean ± SEM.

in the cerebral cortex [16] is due to a competition of exogenously administered GHBA with endogenous GABA for the GABA receptors. This antagonism may explain the GHBA induced myoclonic jerks.

GHBA and GABA competition for GABA-ergic receptors may be the reason for a decrease of GABA inhibitory effect observed in the experiments with a simultaneous microiontophoretic application of both substances [3].

Table 3. – *The effect of GHBA, SSA and AOAA on the survival time and on the survival rate of mices exposed to hypoxic conditions.*

	A Survival time (min)	B Survival rate %
Control	20±1.1	15
GHBA (300 mg/kg)	49±2.2	68
SSA (300 mg/kg)	85±48	60
Valproic ac. (300 mg/kg)	57±5.0	48
AOAA (25 mg/kg)	22±3.1	20

In A mices were placed in a chamber having an initial concentration of O_2 of 8 %. Values are mean ± SEM of at least 20 animals.
In B mices were exposed for 10 min. to a $p_a O_2$ of 35 mmHg.

Conclusions.

The metabolites of the so called « GABA shunt » have several pharmacological properties not related to their transformation into GABA. GHBA in low doses has a tranquilizing effect and, in high doses, a narcotic one; SSA resembles the neuroleptics. Both compounds have a marked protective activity in hypoxic states. Neurophysiological data indicate that they do not potentiate the degree of GABA-ergic inhibition in the cerebral cortex and that in certain conditions they antagonize it. None of them has a protective effect against bicuculline-induced convulsions. Intraventricular administration of GABA and of GABA-mimetics specifically increases GABA-ergic inhibition and manifests antibicuculline action.

The effects of GHBA and SSA are not dependent upon their conversion into GABA. In fact AOAA inhibits their transformation into GABA but does not modify their actions.

Moreover, the electrophysiological experiments reported here suggest that GHBA and SSA are able of antagonizing the action of GABA. The different pharmacological and electrophysiological profile of GABA and GHBA may help to explain the mechanism of actions of valproic acid. This drug may inhibit the metabolism of SSA [17] and therefore it may cause an accumulation of SSA and GHBA. However the possibility of interactions between GHBA and SSA is suggested by electrophysiological experiments supporting the idea that GHBA and GABA interact on the same receptor.

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Pharmacological effects of nicotinamide. Probable endogenous ligand of benzodiazepine receptors

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Summary. – *In the present paper the results are reported of a study of the neuropharmacological effects of nicotinamide (NAM) including the cross-tolerance between NAM, inosine and benzodiazepines.*

Available experimental findings indicate that NAM has sedative, myorelaxant and anticonvulsive effects and affects neurotransmitter turnover as does diazepam.

Riassunto. – *Sono stati studiati gli effetti della nicotinamide (NAM) sul sistema nervoso centrale, comparando la sua azione con quella dell'inosina e delle benzodiazepine. I risultati indicano che la NAM possiede effetti sedativi, miorilassanti ed anticonvulsivanti e influenza il turnover dei neurotrasmettitori in modo analogo al diazepam.*

Introduction.

After the discovery of the benzodiazepine receptors, attempts have been made to identify their endogenous ligands. Presently, four groups of substances capable of specific binding with benzodiazepine receptors have been proposed for this role: purines (inosine, hypoxanthine), β-carbolines, proteins and nicotinamide (NAM). Available experimental findings indicate that the latter has sedative, myorelaxant and anticonvulsive effects and affects neurotransmitter turnover as does diazepam [1–4]. In the present paper the results are reported of a study of the neuropharmacological effects of NAM, including the cross tolerance between NAM, inosine and benzodiazepines.

Methods.

The study was carried out on albino mice (males, 18–24 g) and rats (males, 150–250 g). Tranquillizing effect was estimated using the method of conflict situation in rats, the latter resulting from electric stimulation during the performance of a drinking reflex [5]. The following effects were studied in mice: antiaggressive (fight between two animals on an electrified floor), sedative (disturbed orientation and potentiation of barbiturate sleep), anticonvulsive (antagonism to pentylentetrazol and maximal electroshock seizures), and myorelaxant (motor incoordination in the rota rod); for a detailed description of methods, see [6, 7]. All the substances were injected intraperitoneally, suspended in Tween-80, 30 min before the experiment. Because of the poor penetration of NAM into the brain [8] only high doses (250–2000 mg/kg) were used.

Results and discussion.

NAM in doses of 500–1000 mg/kg caused alterations of the orientation reaction, potentiated barbiturate sleep and had antiaggressive activity. At higher doses, anticonvulsive effects and disturbance of movements were observed (Tab. 1). These effects resemble those of the benzodiazepines [9]; however, different potencies in the different tests were evident: the benzodiazepines had greatest activity in antagonizing convulsions, while the antiaggressive and sedative activities of NAM were more evident.

NAM in doses of 250–500 mg/kg was active in conflict situations, eliminating the action of the punishing factor as shown by the increase in number of drinkings (Tab. 2). A subthreshold dose of NAM (125 mg/kg) was able to potentiate the anxiolytic effect of phenazepam (1) or calcium valproate.

On the other hand, bicuculline, a GABA-ergic receptor blocker, reduced NAM potency. This data provide evidence for an involvement of the GABA-ergic system in the anxiolytic effect of NAM, as is the case of benzodiazepines. As Enna and Snyder [10] have shown, NAM is not capable of binding with GABA-receptor.

To extend our knowledge on the similarity of NAM and inosine to benzodiazepines, cross-tolerance was used. It is known that in man and animals tolerance to the myorelaxant effect of benzodiazepines develops rapidly [11]. The lack of correlation between blood concentration of benzodiazepines or its metabolites and development of tolerance to myorelaxant effect in mice [12] indicates that the mechanism of this tolerance is not related to pharmacokinetic factors. Drawing an analogy with tolerance to morphine, it can be sug-

(1) Phenazepam: 7-bromo-5-(*o*-chlorphenyl)-1,2-dihydro-3H-1,4-benzodiazepine-2-one. Original Soviet tranquilizer.

Table 1. – *Neuropharmacological activity of NAM in mice.*

Method.		
Antagonism to pentylentetrazol	ED_{50} mg/kg	1250 (960–1500)
Antagonism to maximal electroshock	ED_{50} mg/kg	2500 (2000–3125)
Antiaggressive effect	ED_{50} mg/kg	1000 (714–3125)
Disturbance of orientation reactions	ED_{50} mg/kg	500 (357–700)
Disturbance of movement coordination	ED_{50} mg/kg	1600 (1320–1936)
Potentiation of hexobarbital sleep	Sleep duration (min)	
		Control: 29 (16–42)
		NAM 1000 mg/kg: 50 (35–75)

gested that the tolerance to benzodiazepines is underlied by an alteration of the interaction between the drug and the receptor.

Phenazepam in a single high dose (40 mg/kg) caused a marked myorelaxant effect (loss of motor coordination) in 100 % of animals. After a second dose, given 24 hr later, the effect of phenazepam remained in 20 % of mice, after the third one in 5 %, and after the fourth one the effect completely disappeared. In mice tolerant to phenazepam (day 6), lorazepam or diazepam had no myorelaxant effect; after tofizopam myorelaxant effect occurred in only 20 % of animals. In contrast to the benzodiazepines, chlorpromazine, trifluoperazine, ethanol, phenobarbital, meprobamate, hydroxyzine, or the GABA-ergic receptor agonist muscimol given during tolerance to phena-

Table 2. – *Effects of Nicotinamide and other drugs on conflict behavior in rats.*

DRUGS	Doses in mg/kg	Number of approach-with-draw responses	Number of attempts to drink	Motor activity
Control		16,8 11.4÷22,2	2,1 1.5÷ 2,7	24.5 18,2÷30,8
Inosine	250	11 6,6÷15,4	3,7 2.8÷ 4,6	18,5 15,1÷21,9
	500	7,5 4,9÷10,1	15,5 11.8÷19,2	9,2 3,3÷15,1
	1000	2,0 1,3÷ 2,7	6,5 2.1÷10.9	4,0 2,8÷ 5,2
Nicotinamide	125	7,7 4,3÷11,1	5,8 3,9÷ 7,7	10,4 7,6÷13,2
	250	3,4 0,6÷ 6,2	24,5 14,0÷35,0	15,1 8,2÷22,0
	500	4,5 3,5÷ 5,5	32,9 22,1÷43,7	6,9 2,2÷11,6
Phenazepam	0,5	14,5 9,1÷19,9	12,2 10,0÷14,4	3,4 7,7÷14,5
Valproate Ca	200	10,9 4,8÷17,0	15,3 11.6÷19,0	7,6 6,1÷21,3
Diazepam	5	20,8 18,3÷24,3	20,5 14,1÷26,9	23,6 20,5÷26,6
Nicotinamide	125			
+ Phenazepam	+ 0,5	6,2 2,4÷10,0	33,9 27,7÷40,1	13,6 3,0÷24,2
Nicotinamide	125			
+ Valproate Ca	+ 200	11,2 2,9÷19,5	51 12,0÷90,0	32,8 13,2÷78,8
Nicotinamide	250			
+ Bicuculline	+ 1	3,4 0,9÷ 5,9	3,7 1,8÷ 5,6	7,8 4,7÷10,9

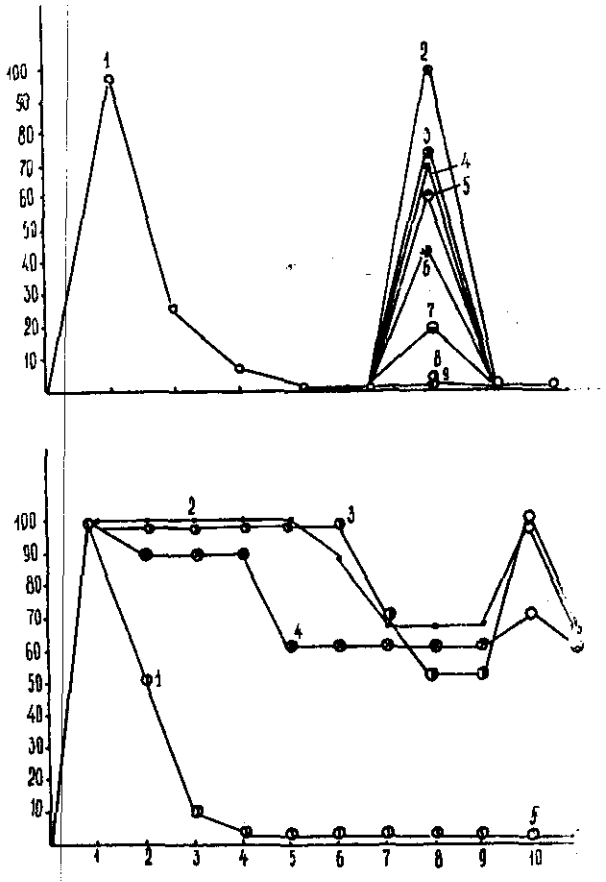

FIG. 1. – Cross tolerance between benzodiazepines and other *Drugs*. *Ordinate:* percent of the effect; abscissa: day of treatment. Upper drawing. Myorelaxant effects of different substances in animals tolerant to phenazepam: 1–○– phenazepam 40 mg/kg; 2–●– meprobamate 200 mg/kg; hydroxyzine 100 mg/kg; chlorpromazine 8 mg/kg; trifluoperazine 4 mg/kg, phenobarbital 100 mg/kg; muscimol 3 mg/kg; 5 –◒– valproate Ca 480 mg/kg; 3 –⊕– inosin 1000 mg/kg; 4 –×– lonetyl 400 mg/kg; 6 –⊗– NAM 2200 mg/kg; 7 –◒– tofizopan (3) 400 mg/kg; 8 –◑– lorazepam 50 mg/kg; 9 –◐– diazepam 40 mg/kg. Lower drawing. Influence of phenazepam upon the tolerance tox other substances. 1 –◍– diazepam; 2 –○– meprobamate; 3 –◑– phenobarbital; 4 –⊗– NAM; 5 –○– phenazepam. The doses are the same as in the upper drawing.

zepam caused a marked myorelaxation in all the animals (Fig. 1). Lonetyl (2), calcium valproate, sodium hydroxybutyrate, inosine and NAM proved capable of partial phenazepam substitution and produced weak myorelaxant effects.

In a second experiment phenazepam was administered after the development of tolerance to other substances (at day 10 of administration), and found inactive in animals tolerant to diazepam and partially active in NAM– tolerant animals, while administered to meprobamate– or phenobarbital–tolerant animals it was followed by myorelaxation in 100 % of mice.

In a third series of experiments, phenazepam was alternated with other drugs. *The administration of* phenazepam to the same animal in turn with meprobamate, hydroxyzine, chlorpromazine, or phenobarbital revealed a differentiation of the effects of the drugs: each compound acted independently, with no connection with each other. Myorelaxant effect of phenazepam under alternating administration disappeared already at day 2–3 of the administration, whereas the effects of chlorpromazine, meprobamate and hydroxyzine remained stable in 100 % of animals even at day 10 (Fig. 2). In contrast, when phenazepam was alternated with diazepam or lorazepam, the action of the compounds was interrelated and the picture of tolerance was the same as under phenazepam alone. NAM and calcium valproate (the latter at the initial stages of tolerance development) had partial cross–tolerance with phenazepam during alternate administration.

These findings suggest specificity of cross tolerance to benzodiazepines. *The compounds which have sufficient affinity for binding with benzodiazepine receptors are those capable of replacing benzodiazepines* (nicotinamide, inosine) [2, 13, 14]. The compounds

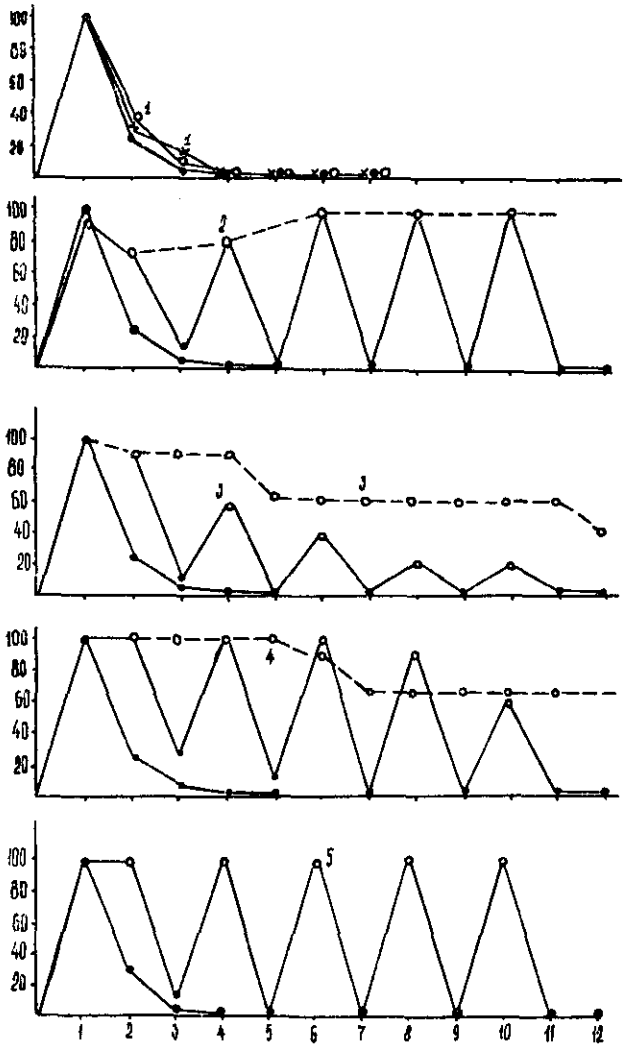

FIG. 2. – Development of tolerance to phenazepam under alternate administration with other psychotropic drugs. Ordinate: percent of the effect; abscissa: day of treatment. Symbols: –●– phenazepam; 1 – diazepam (–○–); or lorazepam (–×–); 2 – valproate Ca (–○–); 3 – NAM (–○–); 4 – meprobamate (–○–); 5 – chlorpromazine (–○–). Doses are as in Fig. 1. Dotted lines indicate the changes in the activity of the compounds (substitutes) during their daily administration to mice in experiments without the alternation with phenazepam.

(2) Lonetyl: 2–methyl–3(n–ethylhydroxyphenyl)–quinazolon–4. Original Bulgarian tranquilizer.

(3) Tofizopam: 1–(3,4–dimethoxyphenyl)–4–methyl–5–ethyl–7,8–dimethoxy–5H–2,3–benzodiazepine. Synonyms: Grandaxine, EGYT–341.

unable to bind (neuroleptics, phenobarbital, non-benzodiazepine tranquilizers, muscimol) [15, 16], do not show cross-tolerance.

Thus, NAM is one of the few endogenous substances capable of specific binding with benzodiazepine receptors and it is similar to benzodiazepines with respect to various manifestations of anxiolytic, hypnotic, and myorelaxant effects. These data, as well as the partial cross-tolerance of NAM and inosine with the benzodiazepines, suggest their resemblance with benzodiazepines and supports the hypothesis of the probable role of these compounds endogenous ligands. The development of tolerance to benzodiazepines does not seem to involve the GABA receptor system, since muscimol, a direct GABA-receptor agonist, does not interfere with the process of tolerance development.

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Influence of cholino-and adrenotropic drugs on immunogenesis

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Summary. – *The present results show the important role of mediators, played at different levels of the neurohumoral maintenance of immune processes. The data on the participation of cholino– and adrenoreactive systems in the modulation of the intensity of immune reaction of the organism point to the importance of drugs in the immunogenesis.*

Riassunto. – *I risultati delle presenti esperienze indicano l'importante ruolo dei mediatori chimici, giocato a vari livelli della regolazione dei processi immunologici. I dati sulla partecipazione dei sistemi colinergici e adrenergici nella modulazione dell'intensità della reazione immunologica indicano l'importanza dei farmaci nell'immunogenesi.*

The central nervous system influences immunogenic processes through hypophysial hormones and the autonomic nervous system [1, 2].

The presence of cholino and adrenoreceptors on the lymphocyte membrane and the possibility of mediator–like substances to influence the functional activity of lymphoid cells has been demonstrated [3–5]. The change in the neuromediator levels in the lymphocyte microenvironment resulting from the activation or inhibition of the corresponding neuro vegetative centers may participate in the regulation of immunocyte functions. In order to analyse the mechanisms modulating the intensity of the immune reaction, we have studied the changes of noradrenaline content in the spleen after antigen administration, the influence of adrenergic agents affecting presynaptic (amphetamine and cocaine) or postsynaptic membranes on the level of 3,5 c–AMP in spleen cells, and the influence of M– and N–cholinergic drugs on the intensity of the rosette–forming cell proliferation. The experiments have been carried out in Wistar rats and CBA mice.

A significant increase of noradrenaline level occurs in the spleen 2 min after the intravenous administration of sheep eritrocytes, this increase is not observed after 10–30–60 min. Noradrenaline level was found increased 24 h later. The noradrenaline level is not increased in the heart after antigen administration and in the spleen after injection of autologous eritrocytes (Fig. 1).

The changes in noradrenaline level are accompanied by a raise in 3,5 c–AMP in the spleen occurring 10 min

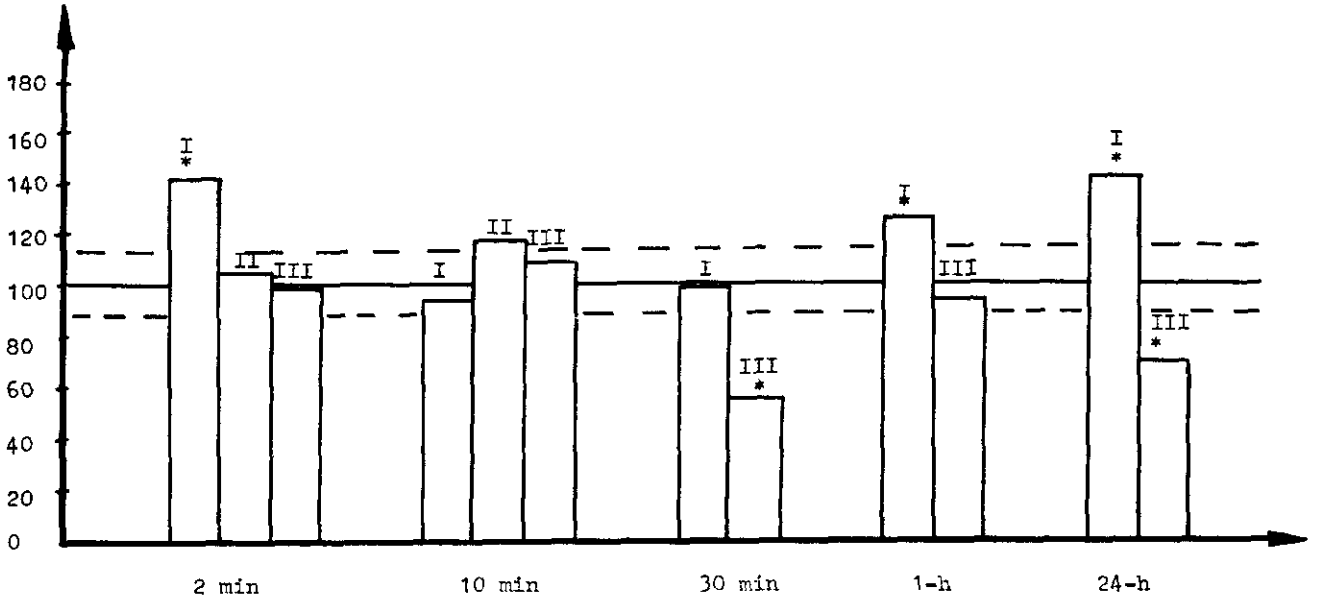

FIG. 1. – Contents of noradrenaline after sheep red blood cells injection in spleen tissue (I), heart tissue (III), and after autologous red blood cells injection in spleen tissue (II). Ordinate = % increase of noradrenaline; Abscissa = time; • = significant difference ($P < 0,05$) as compared with control.

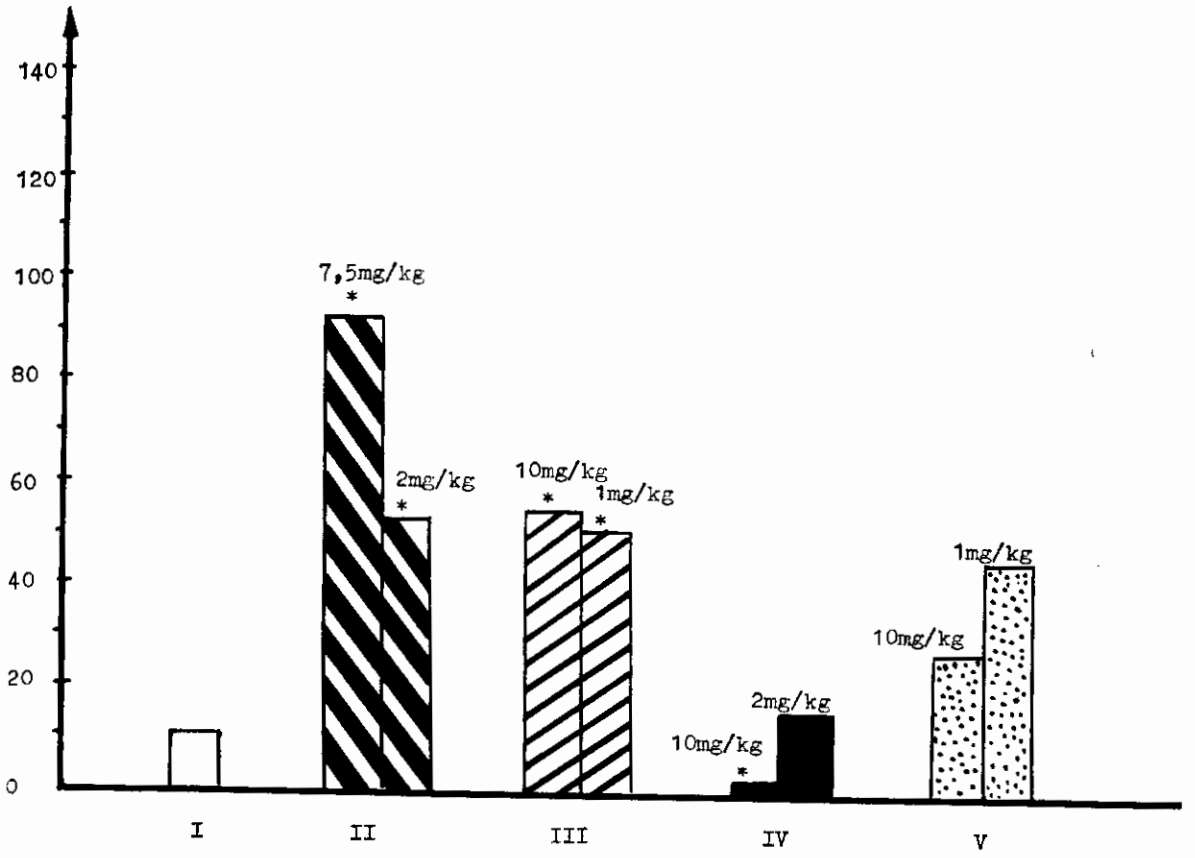

FIG. 2. – Influence of adrenergic drugs on 3,5 c-AMP level in spleen cells. Ordinate = concentration of cyclic AMP (picomoles/10^6 cells); Abscissa = I: control (saline); II: amphetamine; III: cocaine; IV: phenoxybenzamine; V: propranolol; * = significant difference ($P < 0,05$) as compared with control.

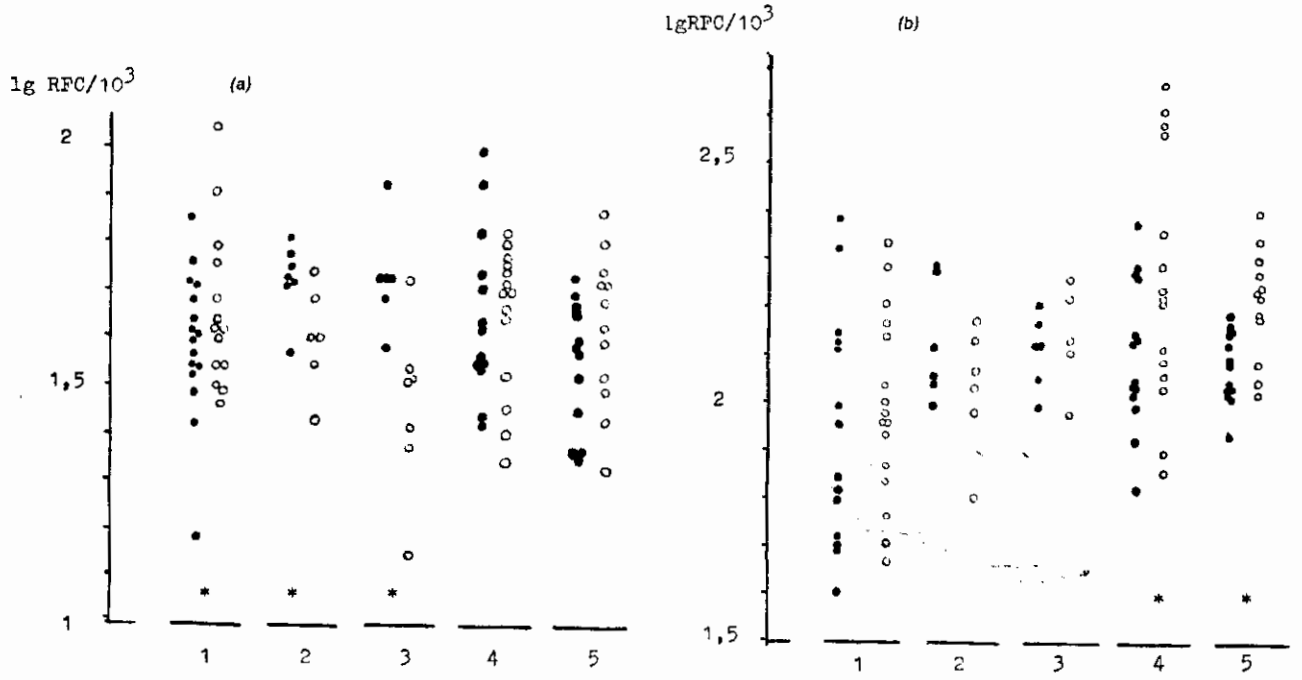

FIG. 3. – Changes induced by injection of cholinergic drugs in the number of rosette forming cells (RFC) at 4 °C (a) and at 37 °C (b) in the spleen of CBA mice on the 6-th day after immunization with sheep erythrocytes. Ordinate = lg RFC per 10^3 spleen cells; white dots: values of each animal treated with drugs; black dots: values of control mice in the same experiments (injection of saline). 1 = nicotine (0,5 mg/kg); 2 = pediphen (1,1-diphenyl-6-diethylaminohex-1-en-4-in dihydrochloride) (25 mg/kg); 3 = benzohexamine (Hesamethonii benzosulfonas) (3 mg/kg); 4 = arecoline (12,5 mg/kg); 5 = pilocarpine (5 mg/kg). * = significant difference as compared with corresponding control in Wilcoxon test.

after the antigen administration and by changes of the functional state of several hypothalamic structures evidenced by shifts in DC potentials [6, 7].

The intraperitoneal injection of amphetamine (7,5 and 2 mg/kg) and cocaine (10 and 1 mg/kg) increases the content of 3,5 c-AMP in spleen cells. Alpha-adrenoblocker phenoxybenzamine (10 and 1 mg/kg) has an opposite effect while the beta-adrenoblocker propanolol (10 and 1 mg/kg) is inactive (Fig. 2).

The participation of the cholinergic system in the maintenance of immune processes has been studied administering cholinergic drugs to mice immunized with sheep eritrocytes repeatedly. N-cholinomimetic drugs lead to an increase in the population of rosette-forming cells, revealed in the spleen at the 6th day after immunization by the rosette formation method at 4 °C. The injection of hexamethonium benzosulfonate (3 mg/kg) and of 1,1-diphenyl-6-diethylaminohex-1-en-4-in, dihydrochloride (25 mg/kg) (N-cholinolytic drugs) leads to a decreased cell population. On the other hand, the administration of M-cholinomimetic drugs arecoline (12,5 mg/kg) and pilocarpine (5 mg/kg) increases the population of rosette-forming cells, revealed by the method at 37 °C, and does not influence the population of rosette-forming cells revealed at 4 °C (Fig. 3).

The increase of noradrenaline levels and of the intracellular 3,5 c-AMP in the spleen following antigen administration suggests that the shifts observed result from the activation of adrenergic mechanism. These effects can hardly be associated with processes developing within the immunocytes, responding specifically to the antigen. They can be attributed to nonspecific components of the reaction to antigens arising in parallel with specific processes; changes in hypothalamic functions are probably one of this components.

The obtained data show the important role of mediators, played at different levels of the neurohumoral maintenance of immune processes. The data on the participation of cholino- and adrenoreactive systems in the modulation of the intensity of immune reaction of the organism point to the importance of drugs in the immunogenesis. The influence of the autonomic vegetative system is directed to the processes of formation of the immunocyte population after antigen stimulation, the character of reconstruction in lymphoid tissue being greatly dependent on a change in balance between sympathetic and parasympathetic system.

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