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Metrazol-Induced Petit Mal: the Role Played by Monoaminergic Mechanisms and Striatum

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ARUSHANIAN, E. B. AND R. M. AVAKIAN. *Metrazol-induced petit mal: the role played by monoaminergic mechanisms and striatum.* PHARMAC. BIOCHEM. BEHAV. 8(2) 113–117, 1978. – Gradually accumulating subconvulsive doses of metrazol give rise to behavioral and electrographic effects close to petit mal epilepsy: slow negative waves and spike-wave complexes on EEG, frozen and myoclonic jerks. Intensification of monoaminergic transmission with apomorphine, DOPA or 5-hydroxytryptophan attenuates, but inhibition (chlorpromazine, haloperidol and p-chlorphenylalanine), on the contrary, increases the subconvulsive effect of the metrazol. Low frequency stimulation of the striatum potentiates, and lesion limits expressiveness of metrazol-induced petit mal. Bilateral electrolytic lesion of the striatum eliminates apomorphine, DOPA and haloperidol action, but slight changes the effects of chlorpromazine, 5-hydroxytryptophan and p-chlorphenylalanine.

Metrazol Corpus striatum Monoaminergic transmission Petit mal epilepsy

SUBCONVULSIVE doses of metrazol given to rabbits evoke EEG-manifestation with EEG-disrhythmic episodes similar to those found in the patients with petit mal epilepsy. Besides, the effect of metrazol displays high sensitivity only towards those anticonvulsants which help in petit mal [12,17]. That's why the changes induced by small doses of metrazol are recognized as an adequate model of this disease.

In recent investigations it was demonstrated that duration of tonico-clonic metrazol-induced convulsions may be changed after modulation activity of central monoaminergic transmission (MA) or corpus striatum [3,4]. It seemed to be interesting to study the role of MA mechanisms and striatum in seizures evoked by the subconvulsive doses of metrazol.

METHOD

Animals

Experiments were performed on 147 adult male nonlinear albino rats weighing 200–300 g. Behavioral and EEG indicies of the preconvulsive state evoked by metrazol were studied before and after injection of MA drugs in the first series of the experiments (94 rats). Some of these animals (15) were used in the second series, when the same indicies were determined by electrical stimulation of the striatum. Effects of metrazol and MA drugs were also obtained during the third series on the brain lesioned (striatectomy – 23 and temporo-occipital cortex lesion (control group) – 15 rats) and shamoperated [10] animals.

Procedures

Under nembutal anesthesia monopolar recording electrodes (platinum, diameter 0.1–0.2 mm) were implanted in the various regions of the brain (ipsilateral – occipital, temporal and frontal cortex, hippocampus, mesencephalic reticular formation; contralateral – striatum). Cortical and subcortical electrical activity was recorded by 4-channel inkwriter electroencephalograph. The experiments were resumed after animals recovery (in a week after operation) and went on for 1–1.5 months.

The stimulation of the brain was carried via chronically implanted electrodes (platinum, diameter 0.2–0.25 mm, distance between tips 0.5 mm). The placement of electrodes into rostral part of the caudate-putamen complex was made stereotaxically by coordinates (AP = 7.8–8.6) of Pellegrino and Cushman [13]. Parameters of the stimulation: rectangular pulses at frequencies of 1, 3, 10 and 30 c/sec, current intencity varied from 5 to 30 μA, pulse duration 0.1 msec. The sites of the electrodes in the brain were verified histologically.

Bilateral electrolytic lesions were made in different brain regions under nembutal anesthesia by using silver electrodes

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(diameter 0.2 mm, direct current 1.5–2.0 mA per 20–30 sec). The animals used in the experiment were 2 weeks after operation. Localization and volumes of the lesions were determined by stereotaxic atlas [13].

Drugs

Metrazol (pentylenetetrazol) was administered intraperitoneally in gradually increasing doses in the form of a 0.05% solution: the first (basal) dose was 10 mg/kg and the others (5 mg/kg) were injected at 5 min intervals up to total dose (20–40 mg/kg), which induced in the rats generalized behavioral and EEG manifestation resembling human myoclonic petit mal. Monoaminergic drugs were injected before metrazol (intraperitoneally, mg/kg): apomorphine – 5 (10 min), L-dioxyphenylalanine/DOPA/ – 100, 5-hydroxytriptophan/5-HTP/ – 100, chlorpromazine – 10, haloperidol – 3 (all these drugs before 30 min), p-chlorophenylalanine (p-CPA) – 600 (48 hr, two days 1X300 mg/kg).

RESULTS

Behavioral and EEG Features of the Preconvulsive Action of the Metrazol

After injection of basal dosage of metrazol (10 mg/kg) in the majority of rats in first 2–3 min locomotor hyperactivity was observed, followed by slight depression. Sedation was intensified by 15–20 mg/kg metrazol and accompanied by periodic typical frozen reaction: during 1–3 sec rats stopped any spontaneous activity and barely visible tremor of the head appeared. At this moment paroxysmal activity which consisted of regular slow negative wave and spike-wave complexes (SWC) with frequency of 3–5 c/sec was observed in various leads of EEG. Agitation, salivation, tachypnea and motor hyperactivity appeared as soon as metrazol was cummulated. But when paroxysmal episodes developed in the EEG, the locomotor activity completely stopped at once.

Another typical index of the metrazol-induced preconvulsive state is the myoclonic jerks (MJ) at the same time with SW activity. The threshold of the MJ is 25. 5 ± 2.4 mg/kg of metrazol. The intensity of the MJ is different: from a slight head twitching to strong jerks in all the body (usually before the attack of convulsions). The MJ are often accompanied by spike-wave complex in EEG. It is necessary to note the close connection between frozen reaction and MJ. The first MJ followed after the frozen reaction and subsequent MJ preceded them. That's why the episodes of the paroxysmal activity consisted of slow negative wave and SWC.

When generalized tonico-clonic attack was approaching, slow negative wave disappeared and SW potentials with predominance of spike component which accompanies strong MJ were recorded. In 2–3 min before the attack correlation between MJ and epileptiform waves was disturbed as a result predominance of paroxysmal activity.

Effect of MA Drugs on the Preconvulsive Action of the Metrazol

Drugs which activated MA transmission by different means (apomorphine, DOPA, 5-HTP) in equal measure prevented the development of preconvulsive effects of the metrazol. Action these drugs did not depend on the fact whether it was accompanied by changes in the spontaneous activity of the rat (motor hyperactivity, stereotype behavior caused by apomorphine and DOPA) or not (after 5-HTP).

In all the cases suppresion of the SW activity and blocking of the frozen reaction and MJ were recorded. After administration of 20 mg/kg metrazol MJ were observed in average only in the 10–20% rats (control – in the 40% rats). The threshold of MJ was also sure to be increased especially after the administration of the DOPA (Table). The number of MJ within preconvulsive state was sharply lowered. For example, for apomorphine its amount is from 9 ± 5 while 56 ± 7 after saline injection. At last their intensity was changed: only strong MJ occured directly before tonico-clonic attack. Epileptiform activity in the EEG was generated only in 4–7 sec before motor manifestation of metrazol-induced fit appeared. This activity consisted mainly of SW discharges with predominance of spike components (Fig. 1). Correlation between MJ and EEG paroxysms was not disturbed.

Blockade of MA transmission by neuroleptics and p-CPA evoked contrary effects. Animals' spontaneous activity after administration of these drugs changed unequally. Chlorpromazine and haloperidol induced motor hypoactivity, drowsiness and synchronization on the EEG whereas p-CPA, quite the reverse, evoked motor hyperactivity and desynchronization on the EEG.

On the background of the inhibitory drugs action just after the injection of the basal dose of metrazol SW activity was developed (Fig. 1). After p-CPA administration epileptiform paroxysms were longer and more frequent. However, the behavioral manifestation didn't appear at this time except some increasing of duration and frequency of the frozen reactions. Doses of metrazol subsequently increasing induced MJ more easily than at the control (Table 1). In the 70–90% rats 20 mg/kg of metrazol evoked MJ Their threshold considerably decreased by p-CPA. Besides the number of MJ within preconvulsive states was increasing. For example, after chlorpromazine the number of MJ averaged 87 (±10). The main rising was accomplished by weak and moderate intensity of MJ. The correlation between MJ and SW activity was disturbed after blockade of MA mechanisms much earlier than in the control.

Influence of the Striatal Stimulation on the Metrazol Effects Changed by Monoaminergic Drugs

According to our previous observations [7] single or low frequencies (1–3 c/sec) of striatal stimulation facilitated the beginning of metrazol-induced frozen reactions, MJ and SW activity. There are optimum conditions for this synergism: with the intensification of the striatal impulses or approach of metrazol doses to the convulsive value both of these influences attenuated one another. Activation of MA transmission by apomorphine, DOPA and 5-HTP accompaned the decrease of metrazol-induced EEG synchronization and simultaneous depression of the striatal effects. On this background the reduced amplitude and duration of the first wave of the striatal evoked potentials in the different brain structures. Common duration of striatal reaction after 20 mg/kg of the metrazol was lower than in the control (Table 1). However with subsequent approach of metrazol doses to convulsive value this antagonism was decreased (Fig. 2). Equally, MA drugs influenced the effects induced by rhythmical (1–3 c/sec) striatal stimulation. Repetitive impulses on the background subconvulsive doses of metrazol evoked afterdischarges which consisted of hyper-

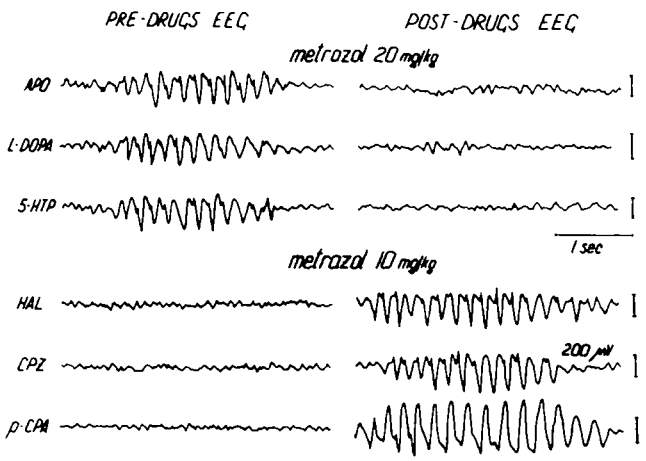

FIG. 1. Different intensity of metrazol-induced SW activity after stimulation and blockade of the central MA transmission. Apomorphine, DOPA and 5-HTP attenuate but haloperidol, chlorpromazine and p-CPA improve the metrazol effects. The leads from frontal cortex in the same rat.

synchronous waves, mainly of the SWC. MA drugs increased the threshold of the striatal-induced paroxysmal afterdischarges: hypersynchronized activity developed after the injection of more higher doses of metrazol than in the control. The epileptiform waves which accompanied the frozen reactions became less distinct.

On the contrary, striatal effects were intensificated by MA transmission blockade. After administration of just 10 mg/kg of metrazol striatal stimulation evoked powerful synchronized responses on the background of neuroleptics and p-CPA. Maximal prolongation of the evoked responses was produced by p-CPA. When metrazol doses approached the convulsive level (30 mg/kg), the striatal reactions were depressed and lowered to the control value. The drugs of this group facilitated also the generation of paroxysmal afterdischarges provoked by low frequency striatal stimulation. Metrazol (20 mg/kg) and repetitive striatal impulses being together triggered in most cases a stable SW activity.

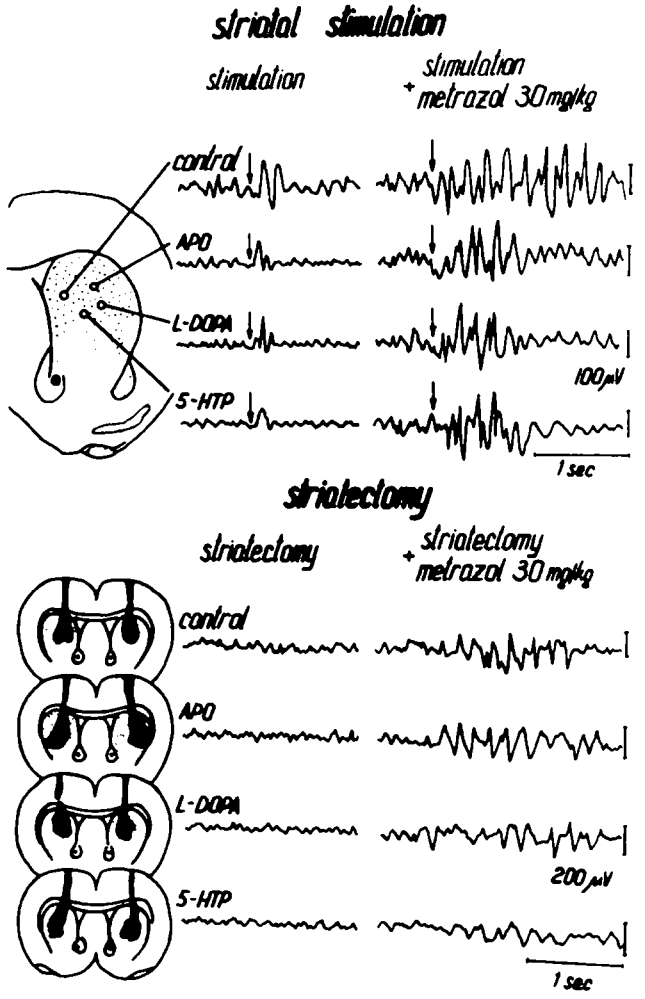

FIG. 2. Modification of the metrazol effects by striatal stimuli (1 c/sec, 8 μA) and lesions in the different rats on the background activation of MA transmission.

TABLE 1

INFLUENCE OF THE MONOAMINERGIC DRUGS ON THE METRAZOL-INDUCED EFFECTS AFTER STIMULATION OR LESION OF THE STRIATUM

DRUGS	N Animals	Duration of the cortical response evoked by single striatal stimulus† (seconds) stimulus	stimulus + metrazol 10 mg/kg	stimulus + metrazol 20 mg/kg	N Animals	Effect of the striatectomy on the myoclonic jerks threshold (metrazol mg/kg) control	N Animals	striatectomy
1. Saline	8	0,48 ± 0,09	0,65 ± 0,12	1,82 ± 0,25	15	25,5 ± 2,4	12	43,8 ± 4,5
2. Apomorphine	6	0,27 ± 0,12*	0,42 ± 0,09*	0,81 ± 0,14*	15	32,5 ± 4,8*	12	48,2 ± 4,9
3. L-DOPA	6	0,21 ± 0,08*	0,39 ± 0,04*	0,76 ± 0,13*	15	35,5 ± 4,1*	12	41,5 ± 5,3
4. 5-HTP	4	0,25 ± 0,13*	0,31 ± 0,12*	0,71 ± 0,18*	10	34,4 ± 2,5*	8	57,5 ± 6,3*
5. Haloperidol	5	0,82 ± 0,13*	1,78 ± 0,19*	2,49 ± 0,21*	15	20,4 ± 3,3	12	40,0 ± 4,2
6. Chlorpromazine	5	0,78 ± 0,17*	1,43 ± 0,13*	2,47 ± 0,23*	15	14,1 ± 2,2*	12	3,12 ± 2,8*
7. p-CPA	4	0,98 ± 0,15*	2,64 ± 0,18*	3,11 ± 0,24*	10	13,0 ± 2,4*	8	26,3 ± 4,5*

*Significant level by $p = 0,05$.

†In all cases was given threshold stimulus (variation of the current intensity 8–15 μA) which evoked in rates sensomotor cortex slow negative waves before drugs administration.

Influence of MA Drugs on the Metrazol Effects in the Rats with Striatectomy

As it was previously reported [6], bilateral lesion of the striatum made production of the effects of the subconvulsive doses of metrazol more difficult. Especially, behavioral manifestation of metrazol induced seizures was sharply disordered. After the operation the MJ threshold was strongly increased (Table 1). The amplitude and duration of the SW activity were lowered after striatectomy. These effects appeared on the background of typical for striatectomized animals motor hyperactivity, high aggressivity and EEG desynchronization.

After striatal lesions protective action of the apomorphine and DOPA on the metrazol-induced petit mal was sharply depressed, but 5-HTP as before the operation considerably blocked SW activity evoked by 30 mg/kg metrazol. Striatectomy also eliminated effect of the haloperidol, although chlorpromazine and especially p-CPA as on the intact rats were decreasing threshold of the MJ and prolonging metrazol-induced spindles.

Localization of the lesions within striatum did not play an important role for the mentioned results. The sizes of lesions played the main role: their critical value which caused the distinct depression of the metrazol-induced petit mal was 7–15% of the volumes of both striata. In cases of small bilateral lesions (3–4% of volume) the reaction of the operated animals on the metrazol and MA drugs was not different from intact and sham operated rats (Fig. 2). Parieto-occipital cortical area lesions at the same parameters of the direct current also no changes during the metrazol-induced petit mal and MA drugs actions.

DISCUSSION

Our data indicate that subconvulsive doses of metrazol evoked outward manifestations are similar to petit mal epilepsy. In this disease in patients the stopping of the movements, myoclonic twitching with a short loss of consciousness and SW potentials in the EEG were observed [11]. Thus our present results give additional grounds to consider that the effects of the subconvulsive doses of the metrazol might estimate as an adequate model of the petit mal.

Intensity of the metrazol action depends on the activity of different brain MA mechanisms. The rise of MJ threshold and blockade of SW rhythmicity may be achieved by nonspecific intensification nor- and dopaminergic transmission (DOPA), selective activation of dopamine receptors (apomorphine) or stimulation of serotoninergic processes (5-HTP). On the contrary the generation of the metrazol-induced MJ and SW activity was facilitated after nonspecific blockade of the catecholaminergic synapses (chlorpromazine), more selective depression of the dopamine receptors (haloperidol) or serotonine synthesis (p-CPA). The action of these drugs on the metrazol-induced effects is determined by changes in the function of different brain structures and principally of striatum, because the activity of this structure distinctly depends on MA mechanisms.

The experiments with stimulation or lesion of the striatum indicate its important role on the metrazol-induced petit mal. The decrease of the MJ threshold, prolongation and facilitation SW activity appearance after low frequency striatal stimulation and, on the contrary, the increase of the MJ threshold and blockade EEG paroxysmal discharges after striatectomy allow us to say that subconvulsive doses of metrazol triggered striatal function.

Striatum may synchronize the cortical activity [15,16]. That is why this structure can be involved in generation of hypersynchronized SW potentials. In particular, SWC provoked in the cats after administration of nembutal-chloralose mixture primarily was observed in the head caudate nucleus – an important part of striatum [14]. From this point paroxysmal waves could be generalized and occupy all cortical areas, apparently through nonspecific thalamo-cortical systems which play a significant role in the genesis of siezures [1].

It was previously shown that metrazol increased caudate synchronization of the cortical activity. The inhibition of the sensomotor cortical neurons activity induced in the cats by single caudate stimuli was rised in the first moment after intravenous infusion of small doses (5–10 mg/kg) of metrazol [5]. And out this fact the opposite synergism is possible: activation of the striatal function must facilitate metrazol evoked effects. Indeed, in the patients with striatal hyperactivity (postencephalic or neuroleptic parkinsonism) threshold of the SW activity generation was two times less than in the healthy men [8].

The action fo the catecholaminergic drugs on the metrazol effects, may be dependent by changes of the striatal function through modification nigro-striatal transmission (apomorphine, haloperidol) and parallel changes in the noradrenergic synapses of brainstem reticular formation (DOPA, chlorpromazine). The reticular activating system playing the role of functional antagonist of striatum [9]. Striatal effects on the metrazol-induced petit mal attenuated by 5-HTP and increased by p-CPA (these facts coincide with the observations of the generalized metrazol seizures model [2,10]. However, after striatectomy effects of serotoninergic drugs on the metrazol induced preconvulsive state had no changes. That is why the action of these drugs may be realized not through striatum, but by means of other brain structures.

The assumed facts allow us to consider that pharmacological modulation of the central MA transmission can be useful for the petit mal epilepsy treatment in clinical practice.

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