Behavioral Changes as a Result of the Diumancal and Dicursinol Influence on the Monoaminergic System of the Rat's Brain

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Acţiunea Diumancalului şi a Decursinolului asupra sistemelor monoaminergice encefalice la şobolan

În experimente pe şobolani masculi a fost studiată acţiunea preparatelor Diumancal şi Decursinol – noi antagonişti de calciu – asupra comportamentului în zona receptorilor noradrenergici presinaptici α_2, stimulaţi de clonidină şi prin epuizarea resurselor de noradrenalină şi dopamină de către alfa-metil tirozină. Diumancalul şi Decursinolul în doze mici reduc hipoactivitatea indusă de clonidină, pe când în doze mari nu potenţează semnificativ sedaţia indusă de clonidină. Uzul combinat al preparatelor nominalizate cu alfa-metil tirozină nu influenţează semnificativ reacţiile de comportament. Rezultatele obţinute sugerează despre rolul autoreglării presinaptice şi al modificării nivelului de neurotransmiţători în mecanismul de acţiune al preparatelor Diumancal şi Decursinol care pot fi utilizate în tratamentul dereglărilor de comportament la unii bolnavi psihici.

Cuvinte-cheie: comportament, sistem noradrenergic, Diumancal, Decursinol.

Действие диуманкала и декурсинола на моноаминергические системы мозга крысы

В опытах на белых крысах (самцых) было изучено влияние новых блокаторов кальциевых каналов – диуманкала и декурсинола на поведенческие реакции на фоне активации пресинаптических норадренергических α_2 – рецепторов клонидином и на фоне истощения лабильных депо норадреналина и дофамина альфа метил тирозином. Было установлено, что оба препарата в малых дозах частично устраняют угнетение поведенческих реакций, вызванных клонидином, а в больших дозах потенцируют угнетение. Комбинированное применение малых доз соответствующих препаратов с α-метил тирозином не выявило изменений поведенческих реакций. Полученные данные дают возможность предположить существование пресинаптической ауторегуляции и лабильного депо нейротрансмиттеров как механизмы действия диуманкала и декурсинола, что может быть использовано в лечении некоторых психических заболеваний.

Ключевые слова: поведенческие реакции, норадренергическая система, диуманкал, декурсинол.

Introduction

Experimental and clinical investigations show that calcium-channel blockers (CCB) have marked psychotropic properties. The profile of their central activity is unique and spans a wide range of effects [4].

In comparison with well-known phenylalkylamin and 1,4-dihydropyridine derivatives, putative new generation calcium antagonists like 2H 1 benzopyrane 2-on derivatives–diumancol and decurcinol are more sensitive to N and T- type voltage-dependent calcium channel proteins [7]. Taking into account the effect of N-type channels as modulation of Ca-depending release of neurotransmitters from the presynaptic membrane, the investigation of presynaptic mechanisms of 2H1 benzopyrane 2-on derivative-CCBs like Diumancal and Decurcinol are a great scientific interest [3].

Diumancal (whose chemical structure is; 7,7-ethyl-endioxy 2 H benzopyrane 2,2 dyon) is used with success in cardiologic practice as an anti-anginal, anti-ischemic and anti-arrhythmic drug. Other investigated the calcium-channel blocker Decurcinol (2,2-dimehtyl-3-oxy-3,-4-dyhidropyrano5,6: 6, 7-coumarin), extracted from the roots of *Ceceli Grandvittatum,* which is endemic to the region and demonstrates markedly anti-arrhythmic effects. Existing evidence claims that Diumancol and Decurcinol have psychopharmacological profiles of action [5].

In view of potential psychotropic activity, the main purpose of the present study is the analysis of the effects of Diumancal and Decurcinol on neuro-mediator processes, and the role of the catecholaminegic system in the action of these compounds.

For the purposes of this experiment, the effects of Diumancol and Decurcinol on behavior were studied on the basis of the pharmacological analyzer α-methyl tyrosine, whose effect on catecholamine metabolism and clonidin is such that it changes the function of presynaptic α-adrenergic receptors. In an attempt to elucidate if a change in NA (noradrenalin) and DA (dopamine) levels were involved in the central action of Diumancol and Decurcinol, as reported in various preclinical studies, monoamines concentrations in three brain structures (striatum, frontal cortex and hypothalamus) were quantified.

Materials and methods

The experiments were performed on 144 male adult rats, in two stages:

- Ist stage – neuropharmacological studies
- IInd stage – neurochemical studies

During the first stage of the evaluation of complex behavioral activity in the animals, the method of "open field" was used. Behavioral reactions—horizontal (the count of passed squares), vertical (the count of standing on hind legs), searching (looking into the holes), grooming (the count of rubbing down and cleaning actions) and defecation (the count of balls) —were observed in the course of 3 minutes. Behavioral reactions were observed 30 minutes after administration, i.e. when the investigated drugs reach their maximal concentra-

tion in CNS. Effective experimental dosages of medications were taken from literature.

Isotonic saline solution and α-methyl tyrosine (200mg/kg) were injected intraperitoneally into the control group and 2nd group of animals, accordingly. α-methyl tyrosine is an inhibitor of tyrosinhydroxilase, which converts tyrosin to DOPA (dihydroxyphenylalanine). It reduces or exhausts the endogen resources of mediators, depending on the size of the dose. The dose that we used of α-methyl tyrosine, caused the reduction of dopamine and norepinefrin levels in the CNS (25-35% and 40-50%, accordingly) 5 hours after administration [6]. Taking this fact into account, 4 hours and 30 minutes after the administration of α-methyl tyrosine, Diumancal (0,1mg/kg) and Decurcinol (1mg/kg) were injected into the 3rd group of animals and after 30 minutes their behavior reactions was observed in the duration of 3 minutes. The 4th group of animals was intraperitoneally administrated clonidin (0,05mg/kg). The 5th group of animals received 0,1; 1 mg/kg Diumancal and 1; 10mg/kg Decurcinol on the background of clonidin, and their behavior was observed within the course of 3 minutes, 30 minutes after admission.

Diumancal, Decurcinol and α-methyl tyrosine were prepared ex *tempore* as a suspension in isotonic saline solution by the addition of Twin-80 in a 200mg/kg dosage. Clonidin was prepared as a suspension in isotonic saline solution in a 0,05mg/kg dosage and administered intraperitoneally.

All chemicals used were of the highest quality available.

During the second stage of experimented, conducted on a basis of Diumancal and Decurcinol pretreatment, NA and DA levels were detected in 3 structures of the rat's brain when compared with control animals.

For the measurement of NA and DA in brain tissue, the single-step method offered by B.M. Kogan and N.V. Nechaev was used [8]. In order to avoid the effects of circadian rhythms to neurotransmitter levels, rats were decapitated at 09:00 a.m. in the laboratory ward, where a temperature of 22°C was maintained. DA fluorescence was measured at a 330 nm excitation wave and 420 nm fluorescence. NA fluorescence was accordingly measured at a 380 nm excitation wave and 478 nm fluorescence. The intensity of DA and NA fluorescence was calculated on the basis of the difference between examination and control tests. The Intensity of fluorophors was analyzed with the "HITACHI"-MDF-4 spectrofluorometer.

Statistical analysis was performed according to the Wilcoxon-Mann-Whitney nonparametric method and on MC EXCELL XP and C-PLUS 2000 computer programs.

Results and Discussions

Initial investigations showed that low doses of diumancal and decurcinol increases the activity of horizontal, vertical and searching reactions, without significant changes in motor activity, and doesn't cause any statistically significant changes in grooming and defecation functions. High doses of investigated calcium antagonists reduce all types of motor activity and cause sedation. These results correspond with the results of other investigators, regarding the reduction of behavioral reactions by calcium antagonists, and confirm the information that Diumancal potentiates the effects of other CNS depressants [9]. The dose-dependent central effects of both medications could be related to the roles of different neuro-mediator systems in the mechanism of action of these medications. In order to find out the relation of stimulating effects of low doses of Diumancal and Decurcinol to catecholaminergic systems, these substances are investigated against the background of α-methyl tyrosine.

As is shown in Table 1, low doses of Diumancal and Decurcinol were completely ineffective in preventing sedative effect of α-methyl tyrosine.

Table 1

Behavioral Reactions Observed During the Combination of Diumancal and Decurcinol with α-methyl tyrosine

Substances	Horizontal activity	Vertical activity	Searching activity	Grooming	Defecation
Control	19,9 (17-22)	2,3 (1-3)	3,1 (2-4)	5,6 (4-7)	1,0 (0-2)
α-MT 200 mg\kg	9,9 (8-12)	1,4 (0-3)	1,6 (1-2)	2,8 (2-4)	0,9 (0-2)
αMT+ diumancal 0,1 mg\kg	10,1 (9-12)	1,5 (1-2)	1,4 (1-2)	2,1 (1-3)	0,5 (0-1)
αMT+ decurcinol 1 mg\kg	10,4 (9-12)	1,6 (1-2)	1,5 (1-2)	2,0 (1-3)	0,9 (0-1)

Note: 1- Control-normal isotonic solution, α-MT - α-methyl tyrosine. 2 - Statistical significance according to Wilcoxon-Mann-Whitney: p < 0,05

Taking into consideration that α-methyl tyrosine has tropism to labile storages of monoamines, it is concluded that labile depots of neurotransmitters play a role in fact that Diumancal and Decurcinol stimulates behavioral reactions in low doses, but doesn't alter this activity when used in combination. On the other hand, the possible modulating effect on presynaptic auto-regulative mechanisms (because of sensitivity to N- and T- type calcium channels) may have a role in the stimulating effect of these medications in low doses.

In order to define in detail the role of presynaptic auto-regulative adrenergic mechanisms in the mechanism of action of diumancal and decursinol, both medications were investigated against the background of clonidin.

It is known that clonidin decreases locomotor activity and other behavioral reactions, which is explained by its stimulating effect on presynaptic α_2 adrenergic receptors and its slowing down of the release of noradrenalin.

Diumancal, administered in doses of 0,1mg/kg, slightly reduced the clonidine-induced hypo activity and sedation in the rats. This effect was more remarkable in horizontal and vertical reactions. Low doses of diumancal restored the activity of horizontal and vertical reactions in 1,3 ($p<0,005$) and 1,5($p<0,05$) times accordingly. There were not any statistically significant changes in defecation, grooming and searching components of behavioral reaction. Despite the fact that lower doses of diumancal were able to antagonize clonidine-induced behavioral inhibition, these indicators were

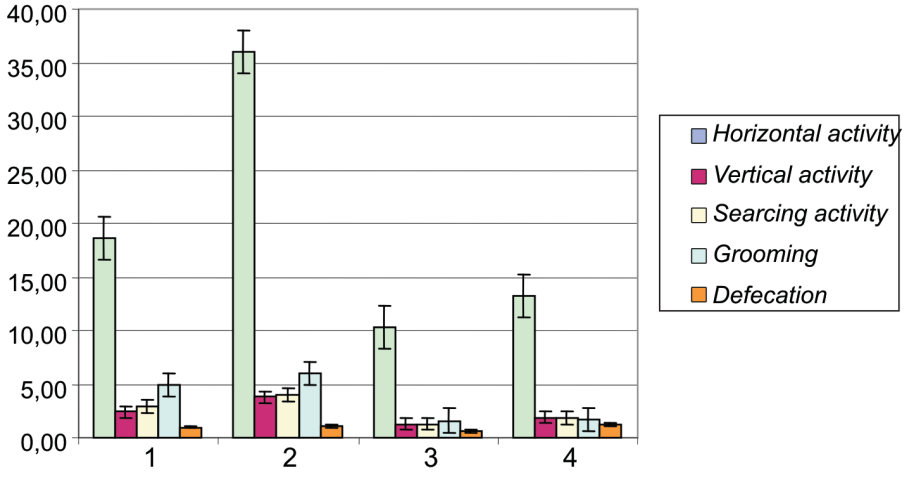

Fig. 1. The Influence of Diumancal to Motor Reactions against a Background of Clonidin.

Note: 1- control group of animal injected normal saline solution; 2- diumancal 0,1mg/kg; 3- clonidin 0,05mg/kg; 4- diumancal 0,1mg/kg+clonidin0,05mg/kg. Statistical significance according to Wilcoxon-Mann-Whitney: horizontal activity p<0,005; vertical activity p <0,05; searching activity p >0,05; grooming p >0,05; defecation p <0,05.

lower than in control animals injected with normal saline solution (Fig.1).

Decurcinol, administered in a 1mg/kg dose, caused a similar effect to Diumancal administered in a 0.1mg/kg dose, but its effect was weak. Antagonism to clonidin low doses of 2H1-benzopyrane 2-on derivatives, may be attributed to the blockade of presynaptic alpha -2-receptors (autoreceptors). These facts confirm the information published in medical literature that claims that some calcium channel blockers blockade presynaptic α_2 adrenergic receptors [1].

On the other hand, the evident stimulating effect of Diumancal and Decurcinol on the background of blockade of presynaptic α_2 adrenergic receptors by clonidin, provides enough of a basis to conclude that other mediator systems have a role in the mechanism of action of these medications, as well.

The behavioral activity of animals receiving low doses of Diumancal and Decurcinol was completely different from the locomotor activity of animals that received high doses of both medications with the combination of clonidin. In both cases, high doses deepen the sedative effect produced by clonidin. Despite the fact that this effect was not statistically clear, the result shows that 2H1-benzopyrane 2-on derivatives influence to CNS is very complicated, and there is a need for broad neuro-pharmacological and even neuro-chemical investigation.

The evidence that CCB-s open these structures in low doses and block in high doses, and besides this, the fact that clonidin blocks N-type calcium channels, explains why diumancal and decurcinol in high doses potentiates the sedative effect of clonidin [2].

In the second stage of present study, the regional concentration of NA and DA were measured on the hypothalamus, striatum and the frontal cortex of rats. The results once again confirmed that CCB's have unique neuro-chemical action. As shown in Table 2, diumancal in a 0,1mg/kg dose decreased the levels of NA in the hypothalamus and frontal cortex, but increased its levels in striatum in 7,6%. Low doses of diumancal increased the levels of DA in the hypothalamus and the frontal cortex in 10,2% and 10,5% accordingly, but decreased its levels in striatum by 9%.

The results of the neuro-chemical investigation of Decurcinol (1mg\kg) were different from those of the Diumancal in a 0,1mg\kg dose. A 1mg\kg dose of Decurcinol significantly decreased NA levels in the hypothalamus and frontal cortex,

Table 2

The Effect of Diumancal, Decurcinol and Verapamil on Levels of Monoamines (ng/g) in Rat's Brains

Substance	Dose mg\kg	Hypothalamus		Striatum		Frontal cortex	
		NA	DA	NA	DA	NA	DA
Control	NaCl 0,9%	689,6 (675-700)	756,6 (740-770)	563,8 (546-580)	642,1 (620-668)	548,1 (520-574)	582,0 (565-590)
Diumancal	0,1	617.8* (605 625)	774.4** (750-790)	606.8* (590-620)	584.3 ** (570-600)	493.6* (478-504)	612.8* (600-624)
Diumancal	1	527.3* (520-538)	683.9* (674-694)	401.6* (388-415)	553.6* (535-570)	416.0* (410-426)	468.6* (460-475)
Decurcinol	1	601.6* (580-625)	689.1* (674-700)	582.9* (546-610)	544.1* (535-550)	456.8* (448-475)	597.1** (510-616)
Decurcinol	10	581.8* (564-596)	613.4* (600-624)	469.5* (460-480)	464.5* (456-475)	445.1* (430-458)	469.0* (460-480)

Statistical significance according to Wilcoxon-Mann-Whitney: *p<0.001; **p<0.05

and increased the striatal NA level 2,2 times less than 0,1 mg\kg Diumancal. Decurcinol (1 mg\kg), but not Diumancal, decreased hypothalamic DA.

The stimulating effect of low doses of Diumancal, in comparison with Decurcinol, is related to the fact that Diumancal severely increases striatal NA and increases the DA level in the hypothalamus differently from decurcinol.

High doses of Diumancal (1mg/kg) and Decurcinol (10mg/kg) decrease the levels of NA and DA in all three brain structures. This fact is a possible explanation for the sedative effect of these medications.

In conclusion, the behavioral and biochemical changes produced by novel CCB-s Diumancal and Decurcinol could be of great interest in light of the possible use of calcium antagonists to treat disorders of the central nervous system.

Conclusions

1. Diumancal and decurcinol were completely ineffective in preventing the sedative effect of α-methyl tyrosine.
2. Both medications, when administered in low doses, slightly reduced clonidine-induced sedation in rats. This effect was more remarkable in horizontal and vertical reactions. There were not any statistically significant changes in the defecation, grooming and searching components of behavioral reactions.
3. Both in high doses deepens clonidin induced sedation, but not in a way that is statistically significant.
4. Diumancal and decurcinol both affect the brain biogenic amines, suggesting both the inhibition and activation of monoaminergic systems, according to dosage.

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Coma hepatică virală B şi/sau D la gravidele cu eclampsie nonconvulsivă

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The Hepatic Coma of Viral Origin B and/or D in Pregnancy and in Eclampsia without Convulsions

The hepatic coma of viral origin B and/or D occurs during all periods of pregnancy but is more common during the last trimester. Pregnancy in the last trimester can be complicated by eclampsia characterized by specific clinical symptoms which evolve into coma. Very few cases are known of a coma in pregnancy which evolved during eclampsia without convulsions. The diagnosis of these cases is difficult. The article describes a coma which evolved during eclampsy without convulsions which was initially believed to be an hepatic coma of viral (B) etiology.

Key words: hepatic viral coma, eclampsia.

Печеночная кома вирусной этиологии В и/или D у беременных с бессудорожной эклампсией

Печеночная кома вирусной этиологии В и/или D не исключается во всех периодах беременности. Беременность в последнем триместре может осложняться эклампсией, протекающей с характерными клиническими симптомами и с развитием комы. Реже печеночная кома у беременных развивается при эклампсии, протекающей без судорог. В подобных случаях дифференциальная диагностика комы сложна. Описывается случай комы при эклампсии без судорог, первоначально принятая как печеночная кома вирусной В этиологии.

Ключевые слова: печеночная кома вирусной этиологии, эклампсия.