

Differential Effects of Systemic Versus Central Routes of Administration of L-Type Calcium Channel Blockers on Pentylenetetrazole-Induced Seizures in Mice

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ABSTRACT:

Differential effects of systemic versus central routes of administration of L-type calcium channel blockers on pentylenetetrazole-induced seizures in mice

Objective: Despite a large number of studies have been reported that L-type voltage dependent calcium channel (VDCC) blockers have anticonvulsant properties, in the vast majority of these experimental studies, VDCC blockers have given through systemic but not central routes of administration. The discrepancies of the anticonvulsant role of VDCC blockers may be due to a potential confounding cardiovascular effect of VDCC blockers, in pretended central action following their systemic administration. In an attempt to clarify such criticism, we examined the effects of three different VDCC blockers such as amlodipine, verapamil and diltiazem on pentylenetetrazole (PTZ)-induced seizures after their systemic intraperitoneal (ip) administration and compared these results with those obtained after their intracerebroventricular (icv) administration in mice.

Methods: Adult male Swiss-Webster mice were used in the study. After 50 min and 30 min following ip and icv. administrations of VDCCBs, respectively, each mouse received a single subcutaneous (sc) injection of 85mg/kg of PTZ and was monitored continuously for 30 min for the appearance of clonic convulsions. The latency of the on-set time of clonic seizures was also recorded.

Results: Ip administration of amlodipine (3 and 5 mg/kg), verapamil and diltiazem (30 and 50 mg/kg) significantly prolonged on-set time of the PTZ-induced seizures. By contrast, icv administration of amlodipine, verapamil and diltiazem (10, 30 and 80mg) had no significant effect on the on-set time of the seizures.

Conclusions: Our results suggest that systemic administered VDCC blockers possess anticonvulsant activity on PTZ-induced seizures. However, lack of anticonvulsant effect of centrally administered L-type VDCC blockers on PTZ-induced seizures indicates that they modulate PTZ-induced seizures by a peripheral, rather than a central mode of action.

Key words: amlodipine, diltiazem, epilepsy, calcium channel, pentylenetetrazole, seizure, mice

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ÖZET:

Farelerde pentilentetrazolle indüklenen epileptik nöbetler üzerine l-tipi kalsiyum kanal blokörlerinin sistemik veya santral verilmesinin değişik etkileri

Amaç: L-tipi voltaja bağımlı kalsiyum kanal blokörlerinin antikonvulsan özelliğinin bulunduğu yönelik birçok çalışma olmasına rağmen bunların çoğunluğunda L-tipi kalsiyum kanal blokörleri santral değil sistemik olarak uygulanmıştır. L-tipi kalsiyum kanal blokörlerinin antikonvulsan rolleri ile ilgili elde edilen farklı sonuçlar onların farklı kardiyovasküler etkilerine bağlı olabilir. Bu nedenle, bu çalışmada, pentilentetrazol (PTZ) ile oluşturulan epileptik nöbetler üzerine değişik L-tipi kalsiyum kanal blokörlerinin etkileri hem sistemik (intraperitoneal, ip) hem de santral (intracerebroventriküler, icv) yollardan verilerek araştırıldı.

Yöntem: Çalışmada Swiss-Webster fareler kullanıldı. Farelere kanal blokerleri ip yoldan verildikten 50 dakika sonra, icv yoldan verildikten 30 dakika sonra subkutan yoldan (sc) 85 mg/kg PTZ enjekte edildi ve denekler 30 dakika süre ile klonik konvülsiyonların gelişimi bakımından izlendi. Herbir denekte nöbetlerin ilk ortaya çıkış süresi de kaydedildi.

Sonuçlar: Ip yoldan verilen amlodipin (3 ve 5 mg/kg), verapamil ve diltiazem (30 and 50 mg/kg) PTZ ile oluşturulan epileptik nöbetlerin başlama zamanlarını geciktirdi. Bunun tersine icv yoldan verilen amlodipin, verapamil ve diltiazem (10, 30 ve 80mg) PTZ ile oluşturulan epileptik nöbetlerin başlama zamanı üzerine herhangi bir etki oluşturmadı.

Tartışma: Bulgularımız PTZ ile oluşturulan epileptik nöbetler üzerine sistemik yoldan verilen L-tipi kalsiyum kanal blokörlerinin antikonvulsan özelliklerinin olduğunu göstermektedir. Bununla beraber, santral olarak verilen L-tipi kalsiyum kanal blokörlerinin PTZ ile oluşturulan epileptik nöbetler üzerine antikonvulsan etki oluşturmaması bu grup ilaçların etkilerinin santral bir etkiden çok periferik olduğuna işaret etmektedir.

Anahtar sözcükler: amlodipine, diltiazem, epilepsi, kalsiyum kanalı, pentilentetrazol, seizure, fareler

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INTRODUCTION

Experimental findings indicated that calcium ions play an important role in the induction and maintenance of seizure activity (1). It is well known that during seizures, the intracellular Ca^{2+} concentration increases and the extracellular Ca^{2+} concentration decreases (2,3). The large

influx of Ca^{2+} is thought to be involved in triggering the burst firing of neurons that occurs during seizures (4). The main route of extracellular Ca^{2+} influx to the cells is VDCCs. Three types of VDCCs (L, N and T-type) with different electrophysiological characteristics and pharmacological sensitivities have been described in neurons (5).

L-type VDCC blockers are widely

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used for the treatment of hypertension. It is well known that L-type VDCC blockers at regular therapeutic doses block Ca^{++} influx into vascular smooth muscle cells, causing vasodilatation and therefore a fall of blood pressure (6). However, it has been suggested that calcium channel blockers agents may play an important role useful in the function of central nervous system by controlling the entry of Ca^{++} into neurons (7-13). Dihydropyridines (eg, amlodipine), phenylalkylamines (eg, verapamil) and benzothiazepines (eg, diltiazem) are different classes of voltage-dependent L-type VDCC blockers that have been demonstrated to interact at distinct sites within the VDCCs (10). It has been suggested that the blockade of the entry of Ca^{2+} into neurons through L-type VDCC may be useful in the treatment of seizures (4). Indeed, the anticonvulsant properties of L-type VDCC blockers have been shown in various models of experimental epilepsy (14). However, there are also contradictory findings in the literature, reporting that some L-type VDCC blockers do not have any antiseizure effect (15) or even they have proconvulsant effects (16-18). Thus, the discrepancies of anticonvulsant role of VDCC blockers may be due to a potential confounding cardiovascular effect of VDCC blockers, in pretended central action of them following their systemic administration (13,19,20). Despite the assertion that L-type VDCC blockers functions as an anticonvulsant due to some experimental studies, in the vast majority of these experimental studies, VDCC blockers have administered through systemic (rather than central) routes of administration.

In an attempt to clarify such criticisms, we examined the effects of three different VDCC blockers such as amlodipine, verapamil and diltiazem after systemic administration on pentylenetetrazole (PTZ)-induced clonic seizures and compared these results with those obtained after their intracerebroventricular administration in mice.

METHODS

Animals and laboratory

All procedures in the present study are in accordance with the Guide for the Care and Use of

Laboratory Animals as adopted by the National Institutes of Health (USA). Adult male Swiss-Webster mice (25-30 g) were used. They were placed in a quiet and temperature- and humidity-controlled room ($22\pm 3^{\circ}\text{C}$ and $60\pm 5\%$, respectively) in which a 12/12 hour light-dark cycle was maintained (08 am - 08 pm light).

Drugs

Verapamil and diltiazem hydrochloride were purchased from Sigma Chemical (USA) and amlodipine besylate was a gift from Pfizer (Turkey). Amlodipine, verapamil and diltiazem were freshly dissolved in saline and given in a volume of 5 ml/kg for i.p. administration.

For i.c.v. injection, verapamil, diltiazem (10, 30 and 80 mg/mouse) and amlodipine (10 mg/mouse) were dissolved in saline. However, due to solubility problem, in high doses of amlodipine (30 and 80 mg/mouse) was dissolved in 20% dimethyl sulfoxide (DMSO) vehicle. Unilateral i.c.v. injections were performed in conscious mice according to the method of Haley and McCormick (1957) (21), using a constant volume of 10 ml/mouse. PTZ was dissolved in saline and administered subcutaneously (s.c.) at a dose of 85 mg/kg.

Experimental Procedure

For studying the convulsing action of PTZ, mice were placed in individual cages and allowed to acclimate to their surroundings. Amlodipine (1, 3 and 5 mg/kg), verapamil and diltiazem (10, 30 and 50 mg/kg) were given i.p. into different groups of mice. Additionally, amlodipine, verapamil and diltiazem (10, 30 and 80 mg/mouse) were given i.c.v. into different groups of mice. Saline was used as a control group for i.p and i.c.v. administration. Additionally, 20% DMSO was used as a control group for high doses of i.c.v. administered amlodipine (30 and 80 mg/mouse). After 50 min and 30 min following i.p. and i.c.v. administration of VDCCBs, respectively, each mouse received a single subcutaneous injection of 85 mg/kg of PTZ and was monitored continuously for 30 min for the appearance of clonic convulsions severe enough to result in posture loss. The latency of the on-set time of clonic seizures was recorded to assess the effects of

treatments on PTZ-induced clonic seizures.

Each animal was used only once and the animals were sacrificed immediately after the end of observation period under ether anesthesia.

Data Analysis

The significance of differences between treatment groups and control groups were analyzed using either one way analysis of variance (ANOVA) followed by Dunnett's test or Chi-square test with one degree of freedom.

RESULTS

The effects of systemic administration of amlodipine, verapamil and diltiazem on pentylenetetrazole-induced clonic seizures:

In saline pretreated mice, PTZ (85 mg/kg, s.c.) produced clonic convulsions resulting in posture loss in mice, with a mean on-set time of 10.2 ± 1.9 min (Table 1). The lowest doses of amlodipine (1 mg/kg, i.p.), verapamil (10 mg/kg, i.p.), and diltiazem (10 mg/kg, i.p.) had no effect on the latency of the on-set time of clonic seizures, but intermediate and higher doses of amlodipine (3 and 5 mg/kg, i.p.), verapamil (30 and 50 mg/kg, i.p.) and diltiazem (30 and 50 mg/kg, i.p.) dose dependently prolonged the latency period of the clonic seizures (Table 1).

Table 1: Effects of intraperitoneal administered amlodipine, verapamil and diltiazem on the pentylenetetrazole-induced (85 mg/kg) clonic seizures

Treatment	Dose (mg/kg)	Subject number	On-set time of the seizures (min)
Saline	-	40	10.2 ± 1.9
Amlodipine	1	12	10.4 ± 1.7
Amlodipine	3	12	$17.7 \pm 1.8^*$
Amlodipine	5	12	$18.4 \pm 1.5^*$
Verapamil	10	12	9.4 ± 1.3
Verapamil	30	12	$18.2 \pm 1.4^*$
Verapamil	50	12	$17.8 \pm 1.3^*$
Diltiazem	10	12	9.7 ± 1.5
Diltiazem	30	12	$14.6 \pm 1.3^*$
Diltiazem	50	12	$15.5 \pm 1.9^*$

Values represent the mean \pm SEM; * $p < 0.05$ as compared to saline treated mice

The effects of i.c.v. administration of amlodipine, verapamil and diltiazem on pentylenetetrazole-

induced clonic seizures:

In i.c.v. saline injected mice, PTZ (85 mg/kg, s.c.) produced clonic convulsions resulting in posture loss in mice, with a mean on-set time of 9.9 ± 1.4 min (Table 2). I.c.v. administration of amlodipine (10, 30 and 80 mg/mouse, i.c.v.), verapamil (10, 30 and 80 mg/mouse, i.c.v.) and diltiazem (10, 30 and 80 mg/mouse, i.c.v.) produced no significant effect on the on-set time of clonic seizures when compared to the saline pretreated group (Table 2). I.c.v. administration of 20% DMSO vehicle had also no effect on the on-set time to PTZ convulsions when compared to saline pretreated group (Table 2).

Table 2: Effects of intracerebroventricular administered amlodipine, verapamil and diltiazem on the pentylenetetrazole-induced (85 mg/kg) clonic seizures

Treatment	Dose (mg)	Subject number	On-set time of the seizures (min)
Saline	-	12	9.9 ± 1.4
DMSO (20 %)	-	12	11.3 ± 1.6
Amlodipine	10mg	12	10.2 ± 1.5
Amlodipine	30mg	12	9.5 ± 0.7
Amlodipine	80mg	12	10.8 ± 1.9
Verapamil	10mg	12	9.8 ± 1.3
Verapamil	30mg	12	13.1 ± 2.9
Verapamil	80mg	12	10.2 ± 2.1
Diltiazem	10mg	12	10.7 ± 1.2
Diltiazem	30mg	12	9.1 ± 1.5
Diltiazem	80mg	12	10.8 ± 1.0

Values represent the mean \pm SEM; DMSO: Dimethyl sulfoxide

DISCUSSION

We provide a comparison between the systemic versus central routes of administration for delivery of VDCC blockers on PTZ-induced convulsions, under the same experimental conditions. Systemic administration of L-type VDCC blockers prolonged to the on-set time of PTZ-induced clonic seizures, whereas i.c.v. administration of L-type VDCC blockers caused no effect on-set time of PTZ-induced clonic seizures. Thus, systemic administered VDCC blockers seem to prolong to the on-set time of PTZ-induced clonic seizures by a peripheral rather than central action.

PTZ is the most frequently used chemoconvulsant in experimental models of epilepsy (22). Most drugs

with anticonvulsant activity in the s.c. PTZ seizure test retard the latency to seizures (22). Thus, dependent on the increase in the seizure latency caused by systemically administered L-type VDCC blockers in PTZ test, it is assumed that L-type VDCC blockers possess anticonvulsant properties in PTZ test. These findings are consistent with previous studies showing that L-type VDCC blockers when given systemically produce an increase in the onset time for convulsions (12). It has been reported that L-type blockers such as the 1,4-dihydropyridines, phenylalkylamines and benzothiazepines are primarily used for treatment of cardiovascular diseases and exert their therapeutic effect via inhibition of vascular L-type Ca^{++} channels. Although these agents bind with high affinity to L-type VDCC, it has been known for many years that they also interact with non-L-type Ca^{++} channel structures. The mechanism by which PTZ elicits its action is not very well understood (23). However, one generally accepted mechanism by which PTZ is believed to exert its action is by acting as an antagonist at GABA-A receptor complex. Interestingly, Das et al recently demonstrated that the inhibitory effects of dihydropyridines on GABA-A receptors (24,25). Thus, such an interaction would explain why proconvulsive effects of VDCC blockers reported in some studies differ from those anticonvulsive effects on pentylenetetrazole test (18). In studies of investigating of central effects of VDCC blockers, their peripheral effects on the cardiovascular system may cause a misinterpretation of central action of VDCCBs. So, it is likely that the reduction in arterial blood pressure following peripheral VDCC blockers administration may readily cause vasodilatation and delay absorption of PTZ from subcutaneous tissue due to reduced tissue perfusion. In consistent with this hypothesis, all systemic administered VDCC blockers with intermediate or higher doses prolonged the on-set time for convulsions to PTZ. Interestingly, the doses of amlodipine that prolonged the latency to the seizures against PTZ were much lower than that verapamil and diltiazem. In supporting our hypothesis, it has been reported the potency of amlodipine was higher than that of verapamil and diltiazem in decreasing blood pressure (10). Thus, it seems likely that the higher potency of amlodipine against pentylenetetrazole

seizures may be due to its higher potency to block vascular L-type VDCCs when compare diltiazem and verapamil. Besides, it has been reported that hydralazine, a non-calcium channel antagonist significantly suppressed the seizures elicited by PTZ (26). Thus, a comparison of the effects of VDCC blockers with those of hydralazine, suggests that the relationship between haemodynamic factors and anticonvulsant action of VDCC blockers on PTZ-induced seizures seem likely.

In contrast to systemic effects of VDCC blockers on PTZ-induced seizure latency, i.c.v. administered VDCC blockers remained without influence on-set time for convulsions to PTZ. Considering these different effect of VDCC blockers following their systemic and central administration, it seems that VDCC blockers modulate seizure activity through their peripheral effects. Thus, it is possible that lack of systemic doses of VDCC blockers was because they were too low to reach high enough to be effective in the central nervous system. This is not likely, since the i.c.v. doses of amlodipine, verapamil and diltiazem, were comparable to those previously reported to be effective in producing central effect such as antinociception (20,27,28). In consistence with our study, L-type VDCC blockers were found to be ineffective on some experimental epilepsy models after i.c.v. administration with comparable doses of used in our study (29,30). When taking into account the lack of action of amlodipine, verapamil and diltiazem after i.c.v. administration on PTZ-induced seizure latency in comparisons to their systemic administration may indicate that L-type VDCC blockers do not have direct effect on brain areas involved in the pathophysiology of PTZ-induced seizures.

In conclusion, our results suggest that systemic administered VDCC blockers possess anticonvulsant activity on PTZ seizures. However, lack of anticonvulsant effect of centrally administered L-type VDCCBs on PTZ-induced clonic seizures indicates that VDCC blockers modulate PTZ-induced clonic seizures by a peripheral, rather than a central mode of action. These finding have important implication that a distinct possibility of the peripheral effects of L-type voltage dependent calcium channel blockers confounding the interpretation of psychopharmacological properties of calcium channel inhibitors.

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