

Effect of Sodium Valproate on Ouabain-Induced Arrhythmia in Isolated Guinea-Pig Atria

ABBAS POUSTI, ALI ZARE JAHROMI, GOLROKH MALIHI, HASAN OJAGHZADEH, KAVEH BRUMAND and TARA DEEMYAD

For author affiliations, see end of text.

Received July 27, 2006; Accepted January 7, 2007

This paper is available online at <http://ijpt.iums.ac.ir>

ABSTRACT

Sodium valproate (SV), an antiepileptic drug has several mechanism of action. It inhibits voltage-sensitive Na^+ channels and reduces intracellular Na^+ accumulation. These actions are similar to that of both phenytoin and carbamazepine. We have investigated the direct cardiac action of SV and its effects on ouabain-induced arrhythmia in isolated guinea-pig atria. The guinea-pig atrium was dissected out and suspended in modified Krebs solution under physiologic conditions. Drug was added into the solution. The changes in rate and contractions were measured using a physiograph. SV (100-300 $\mu\text{g/ml}$) caused a dose-dependent decrease in contractile force of isolated guinea-pig atria (9-120%, $p < 0.001$), but no change on the rate of contractions. Ouabain alone (1.2 $\mu\text{g/ml}$) produced arrhythmia at 10 min and asystole at 21 min. Pretreatment with SV (200 $\mu\text{g/ml}$) significantly increased time of onset of arrhythmia to 29 min and asystole to more than 38 min. SV may have a direct cardiac effect to reduce the membrane conductance through ion channels which may decrease ouabain toxicity in isolated guinea-pig atria.

Keywords: Sodium valproate, Ouabain, Isolated atria

Inhibiting voltage-regulated ion channels is a common mechanism of antiseizure drugs, so some antiepileptic drugs inhibit voltage-activated Na^+ channels and reduce the flow of Ca^{2+} through T-type Ca^{2+} channels [1]. Valproate is a simple monocarboxic acid that is used currently as one of the major antiepileptic drugs with efficacy for treatment of both generalized and partial seizure in adults and children. The drug is increasingly used for treatment of bipolar depressive illness and neuropathic pain and for prevention of migraine attacks. Valproate has several mechanisms of action that is probably contribute to its broad spectrum of antiepileptic effects [2]. It Inhibits voltage-sensitive sodium channels and prolongs recovery of voltage-activated sodium channels from inactivation [1]. It has been reported that valproate has an ability to reduce intracellular Na^+ accumulation in an activity-dependent manner [3]. Valproate increases conductivity of K^+ resulting in neuron hyperpolarization, inhibits N-Methyl-D-aspartate (NMDA)-related transitory polarization in pyramidal cells and reduces NMDA-receptors-stimulated influx of Ca^{2+} into hippocampus cells [4].

The action of valproate is similar to that of phenytoin and carbamazepine [1]. It has been reported that carbamazepine is effective in terminating ventricular

tachyarrhythmia produced by digitalis excess [5]. Thus, the effectiveness of valproate on ouabain-induced arrhythmia may be through similar action.

In the present study, it has attempted to determine whether sodium valproate has an effect in the prevention of ouabain-induced arrhythmia in isolated guinea-pig atria.

MATERIALS AND METHODS

Animals

Guinea-pigs of either sex weighing 450-600g were anaesthetized by ether and exsanguinated. The heart was rapidly removed; the auricles were dissected from the heart and suspended in a bath containing 50 ml of oxygenated modified Krebs solution at 36-37°C, pH 7.4. The composition of solution in mM was as follows: NaCl 118.0, KCl 4.7, CaCl_2 2.6, MgCl_2 1.2, NaH_2PO_4 1.0, NaHCO_3 25.0, glucose 11.1, EDTA 0.004 and ascorbic acid 0.11. After mounting, the preparation was allowed to stand for 30 min for equilibration. Solutions of drugs were prepared so that a constant volume of 0.5 ml for each dose was added to 50 ml of the bathing fluid. The rate and force of spontaneous contractions

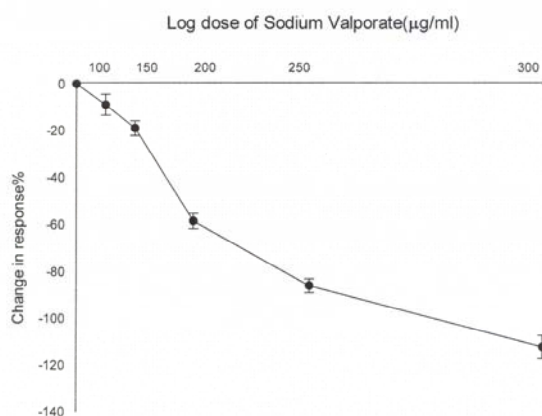


Fig 1. The effect of Sodium Valproate (100,150,200,250,300 µg/ml) on Contractile force of isolated guinea-pig atria. (n=7; Pvalue<0.001)

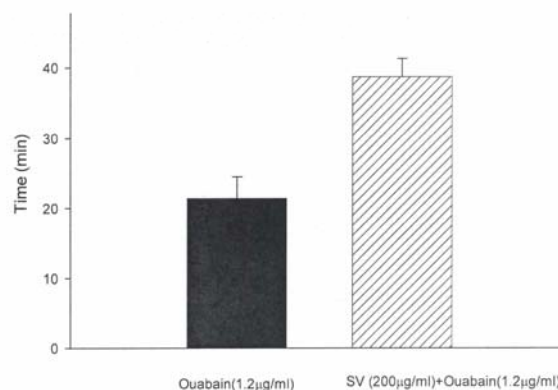


Fig 2. The mean time of asystole (n= 7; Pvalue<0.001)

were recorded isometrically with a photosensitive transducer on a Beckman RS Dynograph recorder. Seven atria were used for each experiment. The duration of spontaneous rhythmicity of guinea-pig atria preparations under our experiments conditions was 20-30 min [6].

Drugs

The following drugs were used: sodium valproate (Rouzdarou Pharmaceutical Co., Tehran, Iran) and ouabain (Sigma, ST.Louis, USA).

Data Analysis

Results were expressed as mean \pm SE. Statistical significance was determined using student's t-test for paired data.

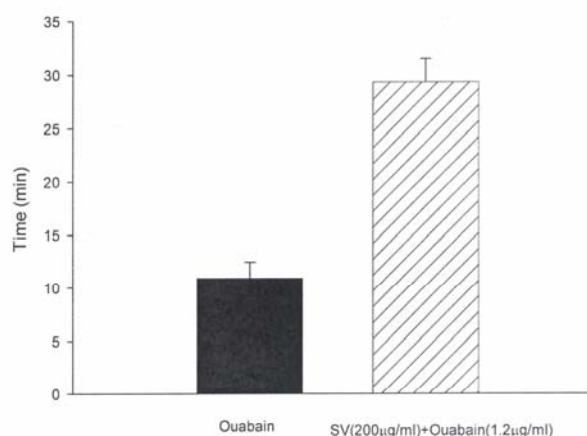


Fig 3. The mean time of arrhythmia (n= 7; Pvalue<0.001, ouabain concentration is equal to 1.2 µg/ml)

RESULTS

Sodium valproate at a concentration of 100-300 µg/ml (n=30) caused a decrease in contractile force (9-

120%) of the isolated guinea-pig atria in a dose-dependent manner ($p<0.001$ Fig.1), but SV had no effect on heart rate when measured for 30 min. SV alone was not arrhythmogenic.

Ouabain 1.2 µg/ml (n=7) alone produced arrhythmia after 10 minutes and asystole at 21 minutes (Fig.2, 3) respectively. Pretreatment with 200 µg/ml SV (n=7) significantly increased the onset of arrhythmia to 29 min ($p<0.001$, Fig.2) and asystole occurred at 38 min ($p<0.001$, Fig.3).The pattern of contractile force by SV +ouabain was more regular than that by ouabain alone.

DISCUSSION

In guinea-pig atria in vitro, sodium valproate produced a significant decrease in the force of contractions ($p<0.001$).The observed effect was dose-dependent. The predominant mechanism of action of sodium valproate described in the literature is its action on ion channels like the other anticonvulsive agents (phenytoin and carbamazepine) [2]. Sodium valproate appears to inhibit the voltage-dependent Na^+ channel [7, 8].

Effects of sodium valproate on potassium and calcium homeostasis in the neurons have been observed. It inhibits calcium influx through NMDA receptors [9]. At high concentration valproate has been shown to increase membrane potassium conductance. Furthermore, low concentrations of valproate tend to hyperpolarize membrane. These findings have lead to speculation that valproate may exert an action through a direct effect on the potassium channels of the membrane [10].

In hippocampal slices, it is suggested that SV interfere with Ca^{2+} entry into nerve ending, reduces the $[\text{Ca}^{2+}]$ and increases K-conductance, which would contribute to a decrease in synaptic transmission [11]. The negative inotropic action of SV in isolated guinea-pig atria is probably attributed to inhibition of cardiac Na^+ and Ca^{2+} channels and hyperpolarization of membrane.

This ionic hypothesis helps to explain our results that SV may produce a direct cardiac action.

In our experiments, SV had no effect on the rate of contractions in isolated guinea-pig atria. This was similar to what observed by other investigators [12]. This effect of SV appears to be mediated through the inhibition of ionic current and probably a non specific effect due to stabilizing action of the membrane. As this molecule at high concentration has highly lipophilic properties, these effects might be the result of depolarization block by direct partition into cell membranes, which alters the transport [13]. This stabilizing action of SV may also help to explain the preventive effect of SV to ouabain-induced arrhythmia in isolated atria.

It has been reported that phenytoin inhibits the intoxication of cardiac tissue induced by ouabain [14]. Cardiac glycoside acts by inhibition of $\text{Na}^+ - \text{K}^+$ pump. Thus, it increases $[\text{Na}^+]_i$ and causing more Ca^{2+} to enter the cardiac myocytes. Digitalis toxicity appears to be caused by excessive Ca^{2+} influx into cardiac cells. In fact, both therapeutic and toxic effects of digitalis are due to myocardial Ca^{2+} loading [15]. In conclusion, the effectiveness of SV in the prevention or delay of ouabain-induced arrhythmia in isolated atria may be through both its phenytoin-like activity and stabilizing action of the membrane which correct the ionic disturbance due to ouabain-toxicity.

ACKNOWLEDGMENT

This work was supported by the Faculty of Pharmacy School at Tehran University of Medical Sciences, I. R. Iran. We also thank Rouzdarou Pharmaceutical Co. in Iran for the generous supply of sodium valproate powder.

REFERENCES

- McNamara JO. Drugs effective in the therapy of the epilepsies. In: Hardman JG, Limbird LE, Eds. Goodman & Gilman's The Pharmacological Basis of Therapeutic, 10th edn. Mc Graw-Hill, New York, NY: 2001; pp 521-536.
- Johannessen CU. Mechanism of action of valproate : a commentary. *Neurochem Int* 2000; 37:103-110.
- El-Mallakh RS, Huff MO. Mood stabilizers and ion regulation. *Harv Rev Psychiatry* 2001; 9:23-32.
- Spasic M, Zivkovic M, Lukic S. Prophylactic treatment of migraine by valproate. *Med Biol* 2003; 10:106-110.
- Steiner C, Wit AL, Weiss MB, Damato AN. The antiarrhythmic actions of carbamazepine (Tegretol). *J Pharmacol Exp Ther* 1970; 173: 3323-3.
- Pousti A, Deemyad T, Malihi G. Mechanism of inhibitory effect of citalopram on isolated guinea-pig atria in relation to adenosine receptor. *Hum Psychopharmacol Clin Exp* 2004; 19:347-350.
- McLean MJ, MacDonald RL. Sodium valproate, but not ethosuximide, produces use-and voltage-dependent limitation of high frequency repetitive firing of action potentials of mouse central neurons in cell culture. *J Pharmacol Exp Ther* 1986; 237:1001-1011.
- Thomas AB, Mathews GC, Ferrendelli JA. Mechanisms of action of antiepileptic drugs. In: Wyllie E, Ed. *The Treatment of Epilepsy: Principles and Practice*, 2nd edn. Williams & Wilkins Baltimore, 1996; p 700-711.
- Page E, Curtis M, Walker M, Hoffman B. *Integrated Pharmacology* 2nd .edn. Moosby Co. UK: 2002; p 255.
- Porter RG, medrum BS. Antiseizure Drugs. In: Katzung BG. *Basic and Clinical Pharmacology*, 9th edn, Mc Graw-Hill, New York, NY, 2004; p 392.
- Franceschetti S, Hamon B, Heineman U. The action of valproate on spontaneous epileptiform activity in the absence of synaptic transmission and on evoked changes in $[\text{Ca}^{2+}]_o$ and $[\text{K}^+]_o$ in the hippocampal slice. *Brain Res* 1986; 368:1-11.
- Lee WS, Limmroth V, Ayata C, Cutrer FM, Waerber C, Yu X, Moskowitz MA. Peripheral GABA receptor-mediated effects of sodium valproate on dural plasma protein extravasation to substance P and trigeminal stimulation. *Br J Pharmacol* 1995; 116: 1661-7.
- Cutrer FM, Limmroth V, Moskowitz MA. Possible mechanisms of valproate in migraine prophylaxis. *Cephalgia* 1997; 17(2):93-100.
- Godfraind T, Lesne M, Pousti A. The action of diphenylhydantoin upon drug binding, ionic effect and inotropic action of ouabain. *Arch Inter de Pharmacodyn et de Therapie* 1971; 191:66-73.
- Brenner GM. *Pharmacology*. 1st ed. W.B. Saunders Co USA: 2000; p115.

CURRENT AUTHOR ADDRESSES

- A. Pousti, Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran. E-mail: drpousti@yahoo.com (Corresponding author)
- A. Zare Jahromi, Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
- G. Malihi, Department of Pharmacology, School of Medicine, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.
- H. Ojaghzadeh, Department of Pharmacology, School of Medicine, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.
- K. Brumand, Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
- T. Deemyad, Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.