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Effect of L-type calcium channel antagonists on spermine-induced CNS excitation in vivo

Karen M. Doyle*, Brian P. Kirby[1], Desiree Murphy, Graham G. Shaw

Department of Pharmacology, School of Pharmacy, Trinity College, Dublin 2, Ireland

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Abstract

The ability of nitrendipine, nisoldipine, verapamil and gabapentin to inhibit the development of CNS excitation induced by spermine was assessed in mice. Injection of an excitotoxic dose of spermine (100 μg, i.c.v.) in mice results in worsening tremor that culminates in the development of a fatal tonic convulsion within 8 h of spermine administration. The dihydropyridines, nitrendipine and nisoldipine, which are L-type calcium channel antagonists acting at the α1 subunit, inhibited the development of spermine-induced effects. Verapamil, which also acts at the α1 subunit of the L-type calcium channel, also inhibited the development of spermine-induced CNS excitation. Gabapentin, a postulated L-type calcium channel antagonist interacting at the α2δ subunit, did not inhibit the development of spermine-induced effects. These results show that antagonists of the α1 subunit of L-type calcium channels can effectively inhibit the effects of spermine in vivo. This may highlight the importance of L-type calcium channels in spermine action.
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The polyamine spermine is a naturally occurring ubiquitous molecule that is necessary for cell growth and differentiation [13,31], and which also may have a neurotransmitter or neuromodulatory role in the CNS [32]. High levels of spermine are toxic to cells, and overactivity of the polyamines has been suggested to contribute to the neuronal damage incurred in many disease states [32]. Exogenous spermine (100 μg, i.c.v.) causes fatal tonic convulsions in mice within 8 h of administration [7]. The mechanisms through which polyamines mediate their effects have not yet been fully elucidated. However, the polyamines have a positive modulatory action on the NMDA receptor through which they mediate some of their excitatory effects [7]. Evidence that the polyamines can modulate various cationic ion channels [23], including L-type calcium channels [22] has also accrued. Calcium influx through voltage-sensitive calcium channels has been implicated in the generation of epileptic activity [26] and neuronal damage in the central nervous system [17,19].

Dihydropyridines, such as nisoldipine and nitrendipine, bind to a site on the α1 subunit of the L-type calcium channel ($Ca_v1.1$–1.4) and prevent calcium influx, thus reducing excitation within cells. Similarly, verapamil binds to the phenylalkylamine binding site on the α1 subunit of the L-type calcium channel and prevents calcium influx. In addition to their clinically useful effects on the cardiovascular system, L-type calcium channel blockers have shown anticonvulsant effects both in the laboratory [4] and in the clinic [3]. Polyamines inhibit diltiazem and nitrendipine binding in the brain [22] and block the inward current activated by the dihydropyridine agonist BAY K 8644 in guinea-pig intestinal smooth muscle [14]. Therefore, it is possible that L-type calcium channels, particularly ($Ca_v1.2$ and 1.3) may be involved in spermine-induced proconvulsive effects.

Gabapentin is a clinically used anticonvulsant drug, that may also have therapeutic potential as an antihyperalgesic agent [10], and in the treatment of affective disorders [18,24].

* Corresponding author. Present address: Physiology Department, National University of Ireland Galway, University Road, Galway, Ireland. Tel.: +353 91493665; fax: +353 91494544.
E-mail address: karen.doyle@nuigalway.ie (K.M. Doyle).
[1] Joint first author. Present address: School of Pharmacy, Royal College of Surgeons, 123 St. Stephens Green, Dublin 2, Ireland.

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Despite being used clinically, the mechanism of action of gabapentin is far from clear. Hypothesised mechanisms such as a direct stimulation of GABAergic receptors (as gabapentin is a cyclohexane derivative of GABA; [20]) and an interaction with the NMDA receptor macrocomplex [29] have been proposed in the past, but subsequently disproved. Excitement over the finding that gabapentin interacts with a high-affinity binding site in rat synaptic plasma membranes led to the proposal that gabapentin-induced effects may be mediated by the large neutral amino acid transporter [16,30]. More promisingly, a high-affinity gabapentin-binding protein has been characterised, and found to be the $\alpha 2\delta$ subunit of the L-type voltage-sensitive calcium channel [12].

The aim of the present study was to investigate the effect of nitrendipine, nisoldipine, verapamil and gabapentin on the development of spermine-induced CNS excitation to assess the involvement of L-type calcium channels in this process.

Female *Laca* mice (20–25 g) were obtained from the Bioresources Unit, Trinity College and were housed in groups of 4–6 under a 12-h light/12-h dark cycle (07:00 h and 19:00 h) with food and water ad libitum. All experiments were conducted according to the requirements of the Cruelty To Animals Act, 1876, European Community Directive, 86/609EC.

Mice given 100 μg of spermine (as spermine tetrahydrochloride), directly into the left cerebral ventricle using the method described by Brittain [1] displayed a distinct behavioural profile of effects (for full description of effects and assessment methods, see [7]). Previous work in this laboratory has demonstrated that there are two temporally and pharmacologically distinct phases of spermine-induced effects [7]. The first phase of spermine effects develops within minutes after spermine injection and generally persists for up to 1 h. Mice show scratching of the upper body, frequent face-washing, and some (∼11%) develop clonic convulsions. This early phase of spermine-induced effects responds to antagonism by a wide range of drugs (e.g. [7,8]). In the present study, nisoldipine, nitrendipine, verapamil and gabapentin all prevented the development of the early spermine effects. The second phase of spermine-induced behaviours seems to show a more selective profile of antagonism, and was the main subject of investigation in this study.

By about 2 h after injection, the second phase of spermine-induced CNS excitation began to develop in the form of body tremor that worsened with time. The CNS excitation culminated in the onset of tonic convulsions, which were ultimately fatal and generally occurred within 8 h of injection.

The methodology used was essentially that described in [7]. Briefly, spermine-induced CNS excitation was assessed at 30-min intervals over a period of up to 8 h after injection. A simple behavioural profile, based on observation, which scored the development of body tremor, and subsequent tonic convulsions was used. The scoring system was as follows: (1) slight tremor; (2) moderate tremor; (3) severe tremor; (4) tonic convulsion-survived; (5) fatal tonic convulsion. The extent of body tremor was assessed by lifting the mouse by the tail and feeling the degree of tremor. The scoring system only recognised three different grades of body tremor in order to reduce scope for assessment error. The assessment was carried out by an experimenter, blind to individual treatment.

Spermine (100 μg) (Sigma, U.K.) was administered as a hydrochloride salt dissolved in 0.9% sterile saline. Nitrendipine (10 mg kg^{-1} or 15 mg kg^{-1}) (RBI, U.K.) was dissolved in 0.9% sterile saline containing the minimum quantity of absolute ethanol for dissolution (3.5% ethanol solution). Nisoldipine (2 mg kg^{-1} or 5 mg kg^{-1}) (Bayer, Germany) was dissolved in 0.9% sterile saline containing the minimum quantity of absolute ethanol for dissolution (5% ethanol solution). Nisoldipine and nitrendipine were unstable in light, and were kept in conditions of darkness during dose preparation. Verapamil (20 mg kg^{-1} or 40 mg kg^{-1}) (Sigma, U.K.) and Gabapentin (100 mg kg^{-1}, 200 mg kg^{-1} or 400 mg kg^{-1}) (Parke-Davis, U.K.) were dissolved in 0.9% sterile saline. All calcium channel antagonists were administered intraperitoneally in a dose volume of 0.1 ml/10 g, 30 min before spermine i.c.v. injection.

The median CNS excitation scores and interquartile ranges (IQR) of the spermine control group and test groups were calculated. Results were expressed in graph form as plots of median CNS excitation score versus time (hours). Statistical significance of the difference between test and control was calculated using the Mann–Whitney *U* test.

In preliminary experiments, doses of nitrendipine (as high as 15 mg kg^{-1}); nisoldipine (up to 5 mg kg^{-1}); verapamil (as high as 40 mg kg^{-1}) and gabapentin (up to 400 mg kg^{-1}) were well tolerated at all doses tested. The doses of nisoldipine, nitrendipine and verapamil used in this study were chosen based on ED_{50} data against NMDA-induced seizures [21]. Doses that exceeded the ED_{50}, but were well tolerated in mice were used. As gabapentin appears to be well tolerated at very high-dose levels, a highest dose of 400 mg kg^{-1} was chosen for gabapentin in this study.

Nitrendipine showed a dose-dependent, statistically significant inhibition of the development of body tremor and tonic convulsions induced by spermine over the observation period (Fig. 1). Nisoldipine also produced a dose-dependent, statistically significant reduction in CNS excitation over the observation period (Fig. 2). Verapamil significantly inhibited the development of spermine-induced CNS excitation, most notably with the highest 40 mg kg^{-1} dose (Fig. 3). However, gabapentin did not inhibit the development of body tremor and tonic convulsions at any dose tested, despite the very high-dose level of drug administered (Fig. 4). In fact, the lowest dose of gabapentin produced a slight enhancement of spermine-induced CNS excitation within 4 h of spermine administration (Fig. 4).

Previous work in this laboratory has demonstrated that there are two temporally and pharmacologically distinct phases of spermine-induced effects [7]. There is evidence suggesting the involvement of the NMDA receptor in both phases of effects [7]. However, polyamines interact at many

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