Br. J. Pharmac. (1985), **84**, 511–515

Interference of sodium with [^{3}H]-nitrendipine binding to cardiac membranes

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1 Interference of sodium with [^{3}H]-nitrendipine binding was studied on membrane homogenates from guinea-pig skeletal muscle, cerebral cortex and left cardiac ventricle.

2 [^{3}H]-nitrendipine binding on homogenates from cerebral cortex and skeletal muscle was unaffected by Na^+ (35 and 140 mEq l^{-1}).

3 On the other hand, for the myocardial receptors, addition of Na^+ resulted in an increase in IC_{50} of nitrendipine.

4 Simultaneously, for the myocardial preparations, saturation curves showed a decrease in [^{3}H]-nitrendipine affinity and an increase in the number of binding sites.

Introduction

Extracellular Ca^{2+} can enter the myocardial cell via various pathways. It is now well known that the conductance channels respond to changes in cellular polarization (voltage-operated channels: VOC) or to receptor activation (receptor-operated channels: ROC). Furthermore, experiments carried out by Reuter & Seitz (1968) and Blaustein & Hodgkin (1968; 1969) have led to the discovery of an exchange diffusion system whereby the influx of Na^+ allows the exit of Ca^{2+}. It has been established that this system can also operate 'in reverse' and mediate the influx of Ca^{2+} into cells (Blaustein & Hodgkin, 1968; 1969). This system seems to be electrogenic, exchanging probably three Na ions for one Ca (Coraboeuf, 1978; Katz *et al.*, 1982; Chapman, 1983). Na^+ and Ca^{2+} channels seem to be distinct, but some authors, like Langer (1974) suggest that 75% of the Na^+ entering the cell during depolarization enters through the so-called 'Ca^{2+} channel'. The discovery of calcium antagonists led to the evidence that Ca^{2+}-dependent cellular processes could be targets for pharmacological agents (Cavero & Spedding, 1983). More recently, optical diffraction techniques have led to a better knowledge of a channel-forming membrane protein (Unwin & Ennis, 1984). Radiolabelled calcium inhibitors ([^{3}H]-nitrendipine, [^{3}H]-nimodipine, [^{3}H]-nifedipine) used as ligands revealed calcium receptors in rat brain (Ehlert *et al.*, 1982a,b; Gould *et al.*, 1982; Marangos *et al.*, 1982), heart (Bellemann *et al.*, 1981; Ehlert *et al.*, 1982a,b; Holck *et al.*, 1982; Janis *et al.*, 1982; Sarmiento *et al.*, 1983) and skeletal muscle (Ferry *et al.*, 1983; Fosset *et al.*, 1983; Glossmann *et al.*, 1983). The inhibition of [^{3}H]-nitrendipine binding by the various di- and tri-valent cations (Co^{2+}, La^{3+}, Mn^{2+}, Ca^{2+}) is consistent with the idea that the binding sites for nitrendipine are associated with calcium channels (Marangos *et al.*, 1982; Fosset *et al.*, 1983). For most authors, these receptors are not influenced by the Na^+ concentration, see Fossett *et al.* (1983) who showed that [^{3}H]-nitrendipine binding in skeletal muscle is not modified by variations in Na^+ concentration. For Ehlert *et al.* (1982b) and Marangos *et al.* (1982), working respectively on rat heart and brain, the addition of Na^+ did not impair [^{3}H]-nitrendipine binding expressed as % of binding without Na^+. In the present work, we demonstrate [^{3}H]-nitrendipine binding on membrane homogenates from guinea-pig skeletal muscle, cerebral cortex and left cardiac ventricle. While Marangos carried out his experiments in the presence of low concentrations of Na^+ (0.1–0.5 and 2 mM), we used physiological concentrations (0.35 and 1.4×10^{-1} M). Moreover, we performed inhibition curves of [^{3}H]-nitrendipine binding by unlabelled nitrendipine, and for left cardiac ventricle, equilibrium saturation curves. Other authors have considered only the maximal specific binding of [^{3}H]-nitrendipine.

Methods

Membrane preparation

Membrane preparation was carried out by the method of Ehlert *et al.* (1982b). Left ventricle, cerebral cortex and skeletal muscle from guinea-pig were removed and homogenized with a polytron in 10 volumes (w/v) of 50 mM Tris-HCl buffer, pH 7.4. The homogenates were filtered through four layers of cheese cloth and centrifuged at 48,000 *g* for 10 min. The pellet was resuspended and centrifuged again five times in the same buffer and at the same speed. The final pellet was resuspended in 50 mM Tris-HCl buffer to give a concentrationn of 1 mg protein ml^{-1}.

Binding assay

One ml of tissue homogenate was incubated with [^{3}H]-nitrendipine (NEN, 80 Ci $mmol^{-1}$) 0.1 nM, and increasing concentrations of unlabelled nitrendipine in 50 mM Tris HCl buffer, pH 7.4 (total volume 2 ml). The binding was investigated both without Na^+ and in the presence of 35 and 140 mEq l^{-1} Na^+ (0.35 and 1.4×10^{-1} M). Choline chloride was used to keep ionic strength constant at Na^+ + choline = 140 mEq l^{-1}. Incubation was carried out for 90 min at 25°C. Membrane-bound [^{3}H]-nitrendipine was trapped by filtration on Whatman GF/B filters. The filters were rinsed with 20 ml 50 mM Tris-HCl buffer, pH 7.4 and transferred to counting vials containing 10 ml scintillation mixture (Packard 299 TM). Radioactivity was measured in a Packard counter at 43% counting efficiency. Binding in the presence of 10^{-5} M nitrendipine was considered as non-specific binding.

For left cardiac ventricle, saturation curves were

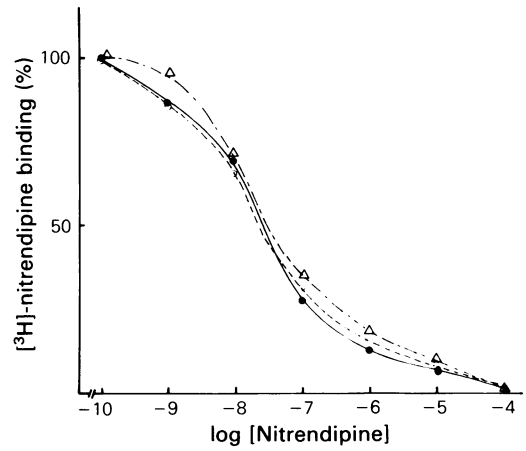

Figure 2 Inhibition of [^{3}H]-nitrendipine binding to skeletal muscle receptors by unlabelled nitrendipine. The inhibition curves were determined without Na^+ (●) in the presence of 35 mEq l^{-1} Na^+ (○) and in the presence of 140 mEq l^{-1} Na^+ (△). Data are means of four determinations, each point being done in triplicate.

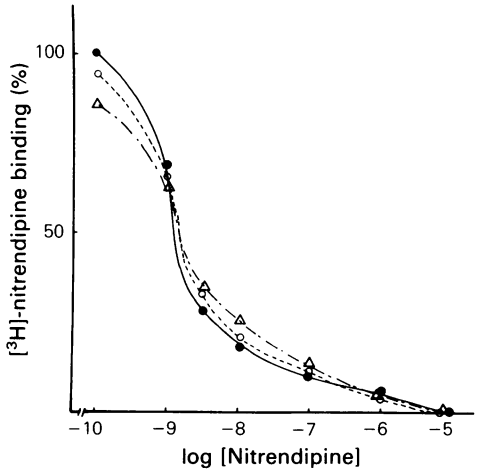

Figure 1 Inhibition of [^{3}H]-nitrendipine binding to cerebral cortex receptors by unlabelled nitrendipine. The inhibition curves were determined without Na^+ (●) in the presence of 35 mEq l^{-1} Na^+ (○) and in the presence of 140 mEq l^{-1} Na^+ (△). Data are means of four determinations, each point being done in triplicate

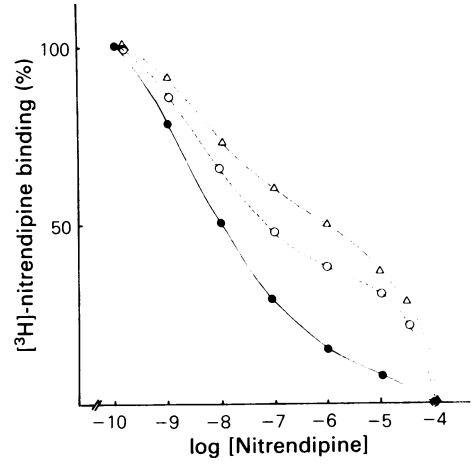

Figure 3 Inhibition of [^{3}H]-nitrendipine binding to myocardial receptors by unlabelled nitrendipine. The inhibition curves were determined without Na^+ (●) in the presence of 35 mEq l^{-1} Na^+ (○) and in the presence of 140 mEq l^{-1} Na^+ (△). Data are means of four determinations, each point being done in triplicate.

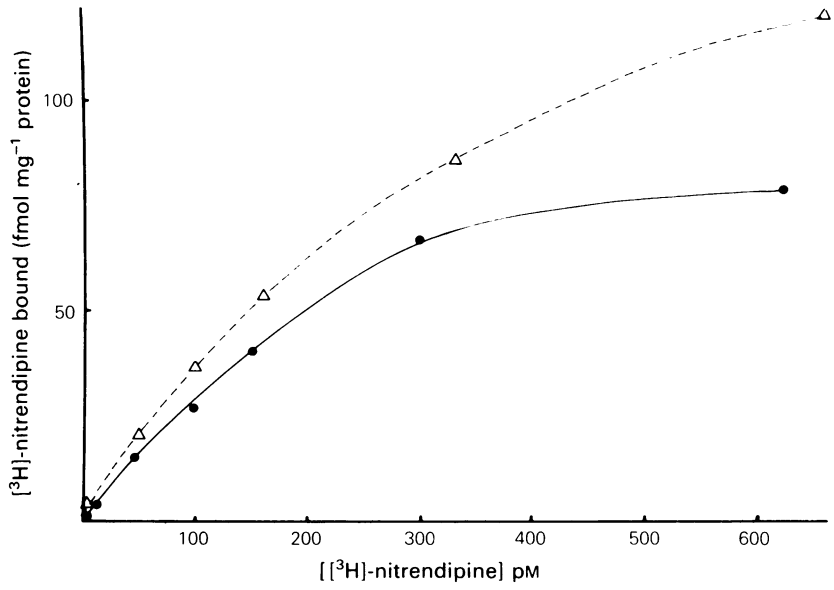

Figure 4 Saturation curve performed in the absence (●) and presence (△) of 140 mEq l^{-1} Na^+. This experiment is representative of five separate determinations.

also performed by incubating 1 ml of tissue homogenate with increasing concentrations of [^{3}H]-nitrendipine (5 to 600 pM) in the absence and in the presence of Na^+. The equilibrium dissociation constant (K_D) and the maximal number of binding sites (B_{max}) were calculated from Scatchard plots.

Results

Inhibition of [^{3}H]-nitrendipine binding by unlabelled nitrendipine on homogenates from guinea-pig cerebral cortex was unchanged in the presence of 35 and 140 mEq Na^+ l^{-1} (Figure 1). The concentration of nitrendipine which caused 50% inhibition of specific [^{3}H]-nitrendipine binding (IC_{50}) was 1.8 ± 0.2 nM without Na^+, 1.9 ± 0.4 nM in the presence of 35 mEq Na^+ l^{-1} and 1.7 ± 0.2 nM in the presence of 140 mEq Na^+ l^{-1}. Similarly, inhibition by unlabelled nitrendipine of [^{3}H]-nitendipine binding performed on homogenates from skeletal muscle was unaffected by the addition of Na^+ (Figure 2). The mean values for IC_{50} were $3.6 \pm 1 \times 10^{-8}$ M without Na^+, $3.3 \pm 0.8 \times 10^{-8}$ M in the presence of 35 mEq Na^+ l^{-1} and $4 \pm 0.7 \times 10^{-8}$ M in the presence of 140 mEq Na^+ l^{-1}. On the other hand, for the left ventricle, the addition of Na^+ resulted in an increase in IC_{50} from $1 \pm 0.6 \times 10^{-8}$ M without Na^+ to $8 \pm 2 \times 10^{-8}$ M in the presence of 35 mEq Na^+ l^{-1} and $1 \pm 0.3 \times 10^{-6}$ M in the presence of 140 mEq Na^+ l^{-1} (Figure 3). In the same way, [^{3}H]-nitrendipine affinity decreased (K_D rose from 0.29 ± 0.04 nM to 0.54 ± 0.04 nM in the presence of 140 mEq Na^+ l^{-1}) whereas the number of binding sites increased from 122 ± 10 to 216 ± 9 fmol mg^{-1} protein (Figures 4 and 5). Differences are significant at $P < 0.01$.

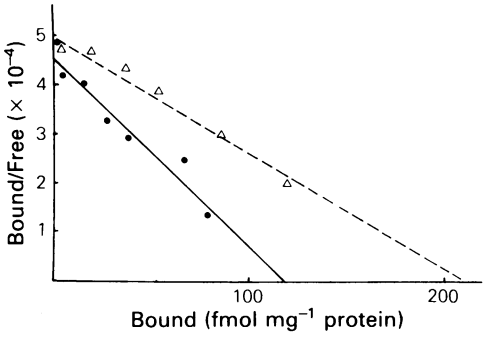

Figure 5 Scatchard plot of the saturation curve described in Figure 4. This experiment is representative of five separate determinations. Without Na^+ (●) mean K_D value = 0.29 ± 0.04 nM and mean B_{max} value: 122 ± 10 fmol mg^{-1} protein. In the presence of Na^+ (△) mean K_D = 0.54 ± 0.04 nM and mean B_{max} value: 216 ± 22 fmol mg^{-1} protein.

Discussion

Ca^{2+} antagonist is a broad term applied to drugs of which the pharmacological effect is to prevent Ca^{2+} from entering the cell. Among these antagonists are the dihydropyridines, which bind on sites associated with calcium channels. Other compounds, such as verapamil, bind on another nearby site causing an allosteric reduction in dihydropyridine binding. Our work shows the dependence of myocardial calcium channels on the Na^+ concentration, but it cannot be assumed that nitrendipine binds on sites other than those related to calcium channels. Consequently, it is probably not at Na^+/Ca^{2+} exchange sites that sodium interferes with nitrendipine binding.

In cerebral cortex or skeletal muscle, Na^+ did not interfere; the singular nature of myocardial contraction suggests regulation mechanisms other than those at work in the skeletal muscle and the central nervous system. Other authors' results apparently contradict ours, but it should be pointed out that Fosset *et al.* (1983) experimented only on skeletal muscle and Marangos *et al.* (1982) only on brain. Ehlert *et al.* (1982b) who worked on heart preparations did not find any effect of Na^+ on [^{3}H]-nitrendipine binding, but they investigated the effect of Na^+ only on maximal specific binding of [^{3}H]-nitrendipine.

The meaning of this phenomenon is debatable. It might cast doubts upon the quantitative evaluation of calcium agonist and antagonist activity by binding techniques. Perhaps too, it might be a crucial phenomenon with a physiological role. On the extracellular side, the Ca^{2+} channel comes into contact with a 140 mEq $Na^+ l^{-1}$ concentration, and, on the intracellular side, with 10 to 30 mEq $Na^+ l^{-1}$ concentrations. Variations in Na^+ flow or concentration in the calcium channels could be a regulating factor, with an increase in Na^+ concentration decreasing the affinity of the protein for calcium.

It should also be recalled that the influence of Na^+ on receptors is not an exceptional phenomenon. Radioligand binding studies have demonstrated that Na^+ can modulate the affinity of adrenoceptors for catecholamines and of other types of receptors (muscarinic, opiate, histamine H_1,) for their respective agonists (Insel & Motulsky, 1984).

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(*Received July 18, 1984.*
Revised September 26, 1984.
Acepted October 15, 1984.)