Effect of Nicorandil upon Different Guinea-pig and Rat Isolated Organ Preparations in vitro

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Summary

A study of the effect of nicorandil (N-2-(hydroxyethyl)nicotinamide nitrate, CAS 65141-46-0), a potassium channel and guanylatecyclase activator, upon preparations of rat vas deferens and uterus, and guinea pig ileum was performed. Nicorandil does not modify rat isolated vas deferens responses to noradrenaline (norepinephrine) and potassium. The drug exerts a non-competitive antagonist effect upon rat isolated uterus response to serotonin, histamine, oxytocin, and, at high concentrations, inhibits guinea-pig isolated ileum responses to acetylcholine, histamine, 4-aminopyridine and potassium.

Zusammenfassung

Einfluß von Nicorandil auf verschiedene Organpräparationen des Meerschweinchens und der Ratte in vitro


Key words CAS 65141-46-0 · Guanylatecyclase activator · Nicorandil, in vitro studies, rat · Potassium channel activator

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2. Material and methods

2.1. General

All experiments were performed using animals housed in our facilities (Faculty of Medicine, Valladolid, Spain) where they were fed ad libitum. They were killed by a blow on the head and exsanguinated. Experiments were performed in the morning and the following drugs were used (pro analysi): acetylcholine hydrochloride, histamine dihydrochloride, atropine sulphate, barium chloride, potassium chloride and nicorandil (M R-503043) (Merk, Darmstadt, Germany), 17-β-estradiol, noradrenaline (norepinephrine) bitartrate, 5-hydroxytryptamine hydrochloride, hexametonium bromide and 4-aminopyridine (Sigma Chemical Co. St Louis, MO, USA), and oxytocin (Sandoz-Pharma, Basel, Switzerland). Carbogen was from Air Liquide (Madrid, Spain). The drugs were dissolved in distilled water and dilutions were made up in saline. Experiments were carried out in isotonic conditions.

2.2. Guinea-pig ileum

The ileum was obtained from male guinea-pigs weighing 300–500 g, and the preparation was set up in accordance with the procedure used in the Department of Pharmacology of the University of Edinburgh [4]. Pieces of 3–5 cm were cut from the ileum between 15 and 30 cm from the ileocaecal junction and suspended under a tension of 1 g in an organ bath (10 ml) containing the incubation medium Tyrode solution (composition, mmol/l: NaCl 139.6; KCl 2.7; CaCl2 3.6; MgCl 2 2.1; NaHCO 3 11.9; NaH2PO4 0.4; and glucose 5.0) gassed with carbogen (95 % O2 /5 % CO2) and maintained at 37 °C. The preparation was allowed to equilibrate (by regular washings) for a period of 30–45 min. When the H1 effect was studied, the incubation medium contained 0.28 mmol/l hexametonium and 10-7 mol/l atropine. Dose-response curves were performed for acetylcholine, histamine, 4-aminopyridine and barium chloride in the absence and presence of nicorandil. Also a sustained contraction was obtained by increasing ten-fold the potassium concentration.

2.3. Rat uterus

The uterine horns were obtained from virgin Wistar rats, with a weight of 150-200 g, in accordance with the procedure of Daly et al. [5]. The animals were treated with 17-β-estradiol (0.5 mg/kg subcutaneously) 24 h before the experiments. Each uterine horn was set up in an organ container with Jalon solution (composition, mmol/l: NaCl 154; KCl 5.6; CaCl2 0.5; NaHCO3 5.9; and glucose 2.8) [6] and suspended under a tension of 1 g in a 10 ml organ bath containing the incubation medium Jalon solution at 31 ± 1 °C. Concentration-response curves to serotonin and histamine were obtained in the presence of nicorandil. Also a sustained contraction was induced by increasing ten-fold the concentration of potassium in the incubation medium [5]. A sustained contraction was induced by increasing ten-fold the concentration of potassium in the incubation medium [5]. A sustained contraction was induced by increasing ten-fold the concentration of potassium in the incubation medium [5]. Also, the preparation was contracted with oxytocin (0.001 IU/ml) and then different concentrations of nicorandil were tested.

2.4. Rat vas deferens

The vas deferens was obtained from male Wistar rats, weighing 150-200 g, in accordance with the procedure of Kitchen [7]. Each vas deferens was set up in an organ container in Krebs-Henseleit solution (composition, mmol/l: NaCl 119; KCl 4.7; CaCl2 2.5; MgSO 4 1.0; NaHCO3 25; KH2PO4 1.2; and glucose 11.1) bubbled with 95 % O2 and 5 % CO2 and maintained at 37 °C. By means of regular washings the preparation was allowed to equilibrate for a period of 30 min. Noradrenaline dose-response curves were generated in the absence and presence of nicorandil. Also a sustained contraction was obtained by increasing ten-fold the potassium concentration.

3. Results

3.1. Guinea-pig ileum

At low concentrations, nicorandil enhanced the effects of acetylcholine and histamine in guinea-pig ileum, while at higher concentrations it shifted the concentration-response curves to the right (Fig. 1).
and 2). This shift was also observed when contracting with 4-aminopyridine (Fig. 3).

Nicorandil potentiates the contractile effect of barium chloride at the lower concentrations tested (Fig. 4) and reduces the contraction induced by potassium ions, being IC₅₀ 4.40 × 10⁻⁴ mol/l with 95% confidence intervals (95% CI) between 1.86 x 10⁻⁴ and 4.80 x 10⁻⁴ mol/l for 12 experiments.

3.2. Rat isolated uterus

Nicorandil produced a shift to the right in the concentration-response curve to serotonin and histamine in rat isolated uterus (Fig. 5 and 6). Also, nicorandil was able to decrease the oxytocin-induced contraction; the IC₅₀ was 5.71 × 10⁻⁴ mol/l with a 95% CI between 4.85 × 10⁻⁴ and 6.68 × 10⁻⁴ mol/l for 16 experiments. Similarly, the drug reduced potassium-induced contraction in rat isolated uterus, though in a dose-independent manner that precluded the calculation of IC₅₀.

3.3. Rat vas deferens

At the concentrations employed, nicorandil did not significantly modify the response to noradrenaline in rat isolated vas deferens, and did not inhibit the contractile effects of potassium ions in the same preparation.

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4. Discussion

At the highest concentrations tested, nicorandil antagonized contractile responses to acetylcholine, histamine and 4-aminopyridine in guinea-pig ileum. Nagai et al. (1991) [8] found that in a similar range (1 µmol/l to 0.1 mmol/l), nicorandil, and other potassium channel activators such as pinacidil and cromakalim, antagonizes histamine-induced contraction of guinea-pig in vitro tracheal preparations, and also exhibits an in vivo efficacy in different experimental asthma models. Sun et al. [99], in guinea-pig ileum, also found that nicorandil, cromakalim and pinacidil were able to inhibit the tonic tension evoked by 30 mmol/l KCl, 0.5 µmol/l histamine or 0.1 µmol/l of oxtremorine in a dose-dependent manner. Thus, our results are in accordance with those reported by these authors in spite of being different experimental models and that a low-doses potentiation is observed. This potentiation emphasizes the results. 4-Aminopyridine is a voltage-dependent potassium channel blocker that facilitates calcium entry and increases acetylcholine release [10, 11]. Its effects are thus antagonized by atropine [12]. In contrast, nicorandil is a potassium channel activator and interferes with 4-aminopyridine induced contraction of guinea-pig isolated ileum. In this sense, its behavior is similar to that of the diazoxide previously studied in our laboratory [12]. On the other hand, Young et al. [13] have proposed that, in guinea-pig ileum, nitric oxide could act at several sites in the intestine through the stimulation of guanylyl cyclase. Also, some guanylate cyclase activator compounds, like sodium nitroprusside, relaxed guinea-pig ileum after the segment had been submaximally contracted by either histamine or acetylcholine [14]. Surprisingly, and unlike papaverine, nicorandil did not antagonize the spasmodic effect of barium chloride. However, papaverine inhibits phosphodiesterase in addition to its calcium channel block effect [15].

The different histamine-effects observed in guinea-pig ileum and rat uterus would be explained by the different histamine receptors involved in the responses, H1 in the ileum and H2 in the uterus. Nicorandil did not modify rat isolated vas deferens response to neither noradrenaline or potassium, although it has been observed to reduce noradrenaline-induced vascular smooth muscle contraction [16]. Differences between the two preparations would account for the different results obtained. Also, Grana et al. [17] found that cromakalim, another potassium channel activator, had no effect over isolated rat vas deferens contracted by noradrenaline. Furthermore, with regard to the nicorandil’s guanylate cyclase activity, Ventura and Burnstock [18] found that L-arginine, sodium nitroprusside and N-nitro-L-arginine methyl ester (L-NAME), all of them with guanylate cyclase activity, did not affect the contractions induced by exogenous application of noradrenaline (10 µmol/l), ATP (1 mmol/l) or BaCl2 (1–10 mmol/l) in rat vas deferens. So, the only possible activity of nicorandil in rat vas deferens would be related to the activity over potassium channels, and like cromakalim, it did not produce any effect over this preparation.

As far as the potassium induced contraction is concerned, nicorandil was unable to relax the vas deferens although did relax guinea-pig ileum and rat uterus. A possible explanation for these discrepancies may be related to the potassium concentration added to contract the preparation; at least this has been observed in rat uterus with some potassium channel activators [19]. On the other hand, it is known that nitric oxide may play a role in that, so the response to nicorandil, a potassium channel activator and guanylate cyclase activator should be complex.

5. References


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