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Phenylpyrazolone derivatives inhibit gastric emptying in rats by a capsaicin-sensitive afferent pathway

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Abstract

Dipyrone (Dp), 4-aminoantipyrine (AA) and antipyrine (At) administered *iv* and Dp administered *icv* delay gastric emptying (GE) in rats. The participation of capsaicin (Cps)-sensitive afferent fibers in this phenomenon was evaluated. Male Wistar rats were pretreated *sc* with Cps (50 mg/kg) or vehicle between the first and second day of life and both groups were submitted to the eye-wiping test. GE was determined in these animals at the age of 8/9 weeks (weight: 200-300 g). Ten minutes before the study, the animals of both groups were treated *iv* with Dp, AA or At (240 µmol/kg), or saline; or treated *icv* with Dp (4 µmol/animal) or saline. GE was determined 10 min after treatment by measuring % gastric retention (GR) of saline labeled with phenol red 10 min after orogastric administration. Percent GR (mean ± SEM, N = 8) in animals pretreated with Cps and treated with Dp, AA or At (35.8 ± 3.2, 35.4 ± 2.2, and 35.6 ± 2%, respectively) did not differ from the GR of saline-treated animals pretreated with vehicle (36.8 ± 2.8%) and was significantly lower than in animals pretreated with vehicle and treated with the drugs (52.1 ± 2.8, 66.2 ± 4, and 55.8 ± 3%, respectively). The effect of *icv* administration of Dp (N = 6) was not modified by pretreatment with Cps (63.3 ± 5.7%) compared to Dp-treated animals pretreated with vehicle (62.3 ± 2.4%). The results suggest the participation of capsaicin-sensitive afferent fibers in the delayed GE induced by *iv* administration of Dp, AA and At, but not of *icv* Dp.

Key words: Gastric emptying; Dipyrone; 4-Aminoantipyrine; Antipyrine; Capsaicin

Introduction

The phenylpyrazolone derivatives dipyrone (Dp), 4-aminoantipyrine (AA) and antipyrine (At) administered intravenously (*iv*; 240 µmol/kg) delay the gastric emptying (GE) of a saline test meal in rats (1-4). Taken together, these studies suggest the participation of the central nervous system (CNS) and of the vagus nerve in the phenomenon. Considering that among the three drugs only Dp induced the same effect when the drugs were administered intracerebroventricularly (*icv*, 4 µmol/animal) and the delay in GE induced by these drugs administered *iv* was abolished by subdiaphragmatic vagotomy (1,3,4), it is possible that the inhibitory stimulus of a drug administered *iv* on GE reaches the CNS through peripheral afferent pathways.

Capsaicin (Cps) is a neurotoxin that, when administered to newborn rats, results in irreversible degeneration of most peripheral afferent neurons with non-myelinated axons (C fibers) and of a minority of scarcely myelinated fibers (Aδ fibers). Capsaicin treatment of newborn rats has been widely used to explore the functional implications of Cps-sensitive afferent neurons. Afferent functions that are impaired by neonatal Cps comprise warmth reception and thermoregulation, cardiovascular reflexes, visceral reflexes, neuroendocrine reflexes, and satiety (5).

In a preliminary study, we demonstrated that the effect of Dp, AA and At administered *iv* on GE was completely blocked in young adult rats pretreated with Cps during the neonatal period, indicating the possibility that afferent fibers sensitive to the neurotoxin may transport the peripheral stimulus that determines the delay of emptying (6).

The objectives of the present study were to determine the effect on GE of *iv* injection of Dp, AA and At at equimolar doses (240 µmol/kg) and of *icv* injection of Dp (4 µmol/animal) in rats pretreated with Cps during the neonatal period.

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Part of these data was presented at the XXI Annual Meeting of Federação de Sociedades de Biologia Experimental (FeSBE), Águas de Lindóia, SP, Brazil, August 23-26, 2006.
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Material and Methods

Male Wistar rats were used. The experimental protocols applied in the present study followed the recommendations of SBCAL (Brazilian Society of Laboratory Animals Sciences) (www.ib.unicamp.br/ceea/principios). Before the surgical procedure for implantation of a cannula in the lateral ventricle of the brain, the animal was sedated with an intraperitoneal (*ip*) injection of thiopental, 75 mg/kg. After cannula implantation or entry into the study, all animals were maintained in individual cages with ration and water available *ad libitum* but they were removed 24 h and 30 min, respectively, before GE evaluation.

Solutions of the drug used for pretreatment, Cps, and the drugs used for treatment, dipyrone, 4-aminoantipyrine and antipyrine (all from Sigma, USA), were prepared on the day of the experiment and protected from light when indicated.

Pretreatment consisted of the subcutaneous (*sc*) injection of Cps at the dose of 50 mg/kg or of vehicle (10% ethanol, 10% Tween 80 in saline) between the first and second day of life (7). After pretreatment the animals were kept in the laboratory, fed by their dams (4 animals per dam) for 4 weeks, and then for an additional 4/5 weeks after weaning, receiving ration and water *ad libitum* in individual cages. To determine the neurotoxic effect of Cps, sensitivity to pain was assessed on the day preceding the determination of GE by the eye-wiping test in both groups using a 1% NaOH solution as an eye irritant (8).

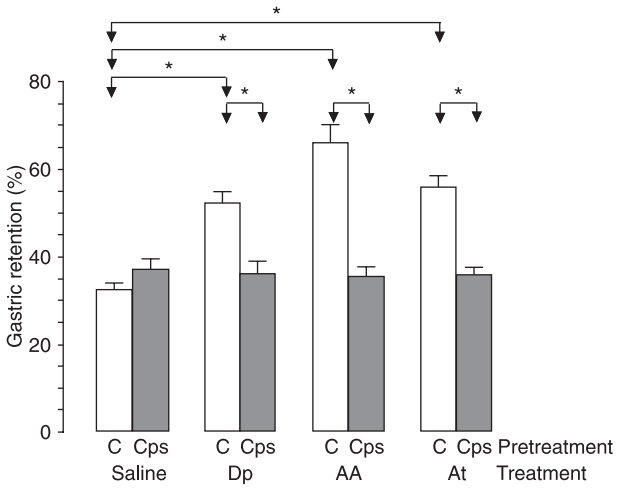

Figure 1. Gastric retention of a saline test meal 10 min after administration to rats by gavage. The animals were pretreated with 50 mg/kg body weight capsaicin (Cps) or vehicle (C) between the first and second day of life. Eight weeks later, 10 min before the determination of gastric emptying, they were treated *iv* with saline or equivalent doses of 240 µmol/kg dipyrone (80 mg/kg; Dp), 4-aminoantipyrine (48.77 mg/kg; AA), or antipyrine (45.17 mg/kg; At). Data are reported as means ± SEM for 8 animals per group. *P < 0.05 (Tukey test).

The GE study was conducted on these animals at the age of 8/9 weeks when they weighed 200-300 g. Ten minutes before the study of GE the two groups were treated *iv* with equivalent doses of 240 µmol/kg Dp (80 mg/kg), AA (48.77 mg/kg), At (45.17 mg/kg), or saline, as described in previous studies (1-4).

Eight days before the study of the effect of *icv* injected Dp, each animal in the groups pretreated with Cps during the neonatal period or with vehicle was implanted with a cannula in the right lateral ventricle using previously established coordinates (1). In the *icv* treatment, 10 µL saline or 4 µmol Dp (1333.2 µg) was used for each animal in the two groups and GE was evaluated 10 min after the end of the *icv* administration, as described in a previous study (1).

GE was assessed indirectly in awake animals by determining the percent gastric retention (% GR) of a test meal of saline labeled with phenol red (60 µg/mL) in a volume of 2 mL/100 g rat weight administered by gavage according to a standardized technique (9). Two modifications were introduced in the methodology previously used: 1) the reading for the determination of the concentration of phenol red dye was performed with a spectrophotometer at 560 nm and 2) before sacrifice the animals were sedated with halothane.

At the end of the experiment all animals were sacrificed and 10 µL 1% Evans blue solution was injected by the same route in the animals that had received the *icv* injection. The brains of the animals in this group were removed, fixed in 10% formalin for 24 h and cut into coronal sections, and the site of the *icv* injection was confirmed when the dye was detected in the IV ventricle.

Data are reported as means ± SEM and were analyzed statistically by ANOVA followed by the Tukey test, when necessary. The level of significance was set at $\alpha = 5\%$ for both tests.

Results

In the eye-wiping test, the group of animals pretreated with Cps during the neonatal period presented 0-3 eye wipes in 10 s (median = 0, N = 44) in order to counteract the irritating effect of a 1% NaOH solution and the group pretreated with vehicle presented 7-14 eye wipes in 10 s (median = 10, N = 44). The animals pretreated with Cps that did not present eye wipes (<4) in 10 s (8) were excluded from the study. The application of this functional test permitted the formation of two well distinct groups regarding the destruction of Cps-sensitive fibers.

Percent GR (mean ± SEM, N = 8) was significantly higher in the animals pretreated with vehicle and treated with Dp, AA, or At (52.1 ± 2.8, 66.2 ± 4, and 55.8 ± 3%, respectively) compared to saline-treated controls (32.2 ± 1.8%; Figure 1), indicating delayed GE of the test meal induced by these phenylpyrazolone derivatives. Animals pretreated with Cps

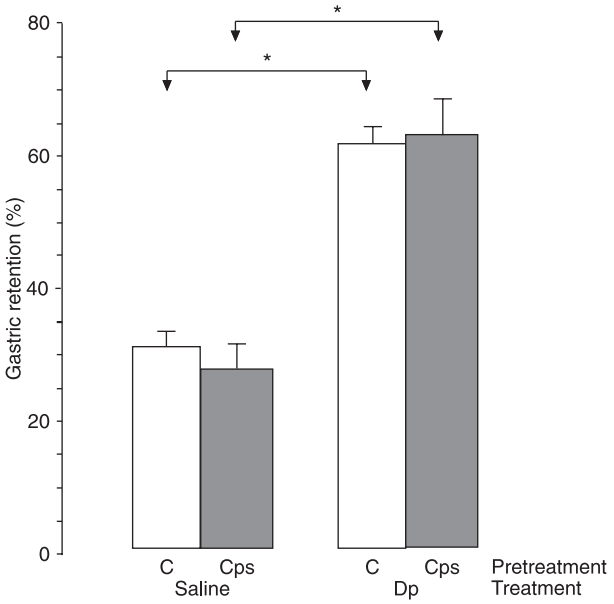

Figure 2. Gastric retention of a saline test meal 10 min after administration to rats by gavage. The animals were pretreated with 50 mg/kg body weight capsaicin (Cps) or vehicle (C) between the first and second day of life. Eight weeks later, 10 min before the determination of gastric emptying, they were treated *icv* with saline or 4 µmol (1333.2 µg/animal) dipyrone (Dp). Data are reported as means ± SEM for 6 animals per group. *P < 0.05 (Tukey test).

and treated with Dp, AA, or At presented GR values (35.8 ± 3.2, 35.4 ± 2.2, and 35.6 ± 2%, respectively) that did not differ from their respective saline-treated controls (36.8 ± 2.8%) but were significantly lower compared to their vehicle-pretreated controls that received the drugs. There was no significant difference between animals pretreated with vehicle or Cps and treated with saline.

Gastric retention data reported as percent (mean ± SEM, N = 6) after *icv* administration of Dp and saline are presented in Figure 2. Treatment with Dp of animals pretreated with vehicle caused significantly greater GR (62.3 ± 2.4%) and Cps (63.3 ± 5.7%) compared to their respective controls vehicle + saline (31.2 ± 2.4%) and Cps + saline (27.7 ± 3.9%). There was no significant difference between the vehicle-pretreated and Cps-pretreated groups that received the same treatment.

Discussion

In the present study, we chose permanent injury to afferent pathways during the neonatal period. This option was based on our observation that the preparation of adult animals (administration of atropine and sedation) for *sc* injection of Cps (a total of 125 mg/kg) at fractionated doses over a period of 2-3 days (5,7) delayed GE *per se*, impairing the assessment of the effect of the drugs.

The results demonstrating the abolition of the effect of *iv* administration of Dp, AA and At on GE in animals pretreated with Cps (Figure 1) suggest the participation of afferent fibers sensitive to this neurotoxin in the transport of the peripheral stimulus that induces delayed GE. This fact permits us to conclude that, when the three drugs are administered *iv*, the phenomenon is not a consequence of their direct action on the smooth muscle fibers of the stomach or on the CNS. Previous studies had already shown that *icv* injection of AA and At does not interfere with GE of a saline test meal (1,3,4), indicating that these two phenylpyrazolone derivatives do not have a central effect.

Dipyrone was the only drug with an effect on GE when administered *icv* (1,3,4), a phenomenon that was not modified in the present study with the destruction of afferent fibers (Figure 2). This result and the observation that the effect of Dp administered *iv* was abolished by the destruction of afferent fibers by Cps make it questionable that the drug administered *iv* can induce delayed GE by a direct action on the CNS as we had previously proposed (1). Thus, the mechanism involved in the effect of Dp administered *icv* probably differs from that involved in the effect of the drug administered *iv*.

In this model, permanent injury to the afferent fibers occurs indistinctly in the extrinsic nervous system (vagus nerve and fibers of spinal projection) (7,10). If we assume that the afferent stimulus, which determines the effect of these three drugs on GE, originates in the digestive tract, it is not possible to determine the pathway it follows.

Section of afferent and efferent fibers by subdiaphragmatic vagotomy abolished the effect of these drugs (1,3,4). Cholecystokinin (CCK) activates the CCK_1 receptors on the Cps-sensitive vagal afferent nerve endings of the intestine and participates in the mechanism of GE control (11,12). 5-Hydroxytryptamine (5-HT; serotonin) also participates in the mechanism of GE control, activating 5-HT_3 receptors on the afferent spinal and vagal nerve endings (13). On this basis, it is tempting to speculate that CCK and 5-HT, which participate in the feedback mechanism of GE by activating the respective receptors on the afferent nerve endings of the vagus nerve, may be involved in this effect. In a preliminary study, we demonstrated that *iv* pretreatment with the doses of devazepide (1 mg/kg) or ondansetron (1 mg/kg) reported in the literature under other experimental conditions (14,15) to be effective for the selective blockade of CCK_1 and 5-HT_3 receptors, respectively, did not abolish or attenuate the delay in GE induced by *iv* administration of Dp, AA and At (16,17). This observation suggests that these receptors may not be involved in the effect of these phenylpyrazolone derivatives on GE.

The observation of the participation of Cps-sensitive afferent fibers in the effect of these drugs on GE and the possible mechanisms involved raise additional speculations, which are outside the scope of the present communication.

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