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Research article

The effects of isatin (indole-2, 3-dione) on pituitary adenylate cyclase-activating polypeptide-induced hyperthermia in rats

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Abstract

Background: Previous studies have demonstrated that centrally administered natriuretic peptides and pituitary adenylate cyclase-activating polypeptide-38 (PACAP-38) have hyperthermic properties. Isatin (indole-2, 3-dione) is an endogenous indole that has previously been found to inhibit hyperthermic effects of natriuretic peptides. In this study the aim was to investigate the effects of isatin on thermoregulatory actions of PACAP-38, in rats.

Results: One μg intracerebroventricular (icv.) injection of PACAP-38 had hyperthermic effect in male, Wistar rats, with an onset of the effect at 2 h and a decline by the 6th h after administration. Intraperitoneal (ip.) injection of different doses of isatin (25-50 mg/kg) significantly decreased the hyperthermic effect of 1 μg PACAP-38 (icv.), whereas 12.5 mg/kg isatin (ip.) had no inhibiting effect. Isatin alone did not modify the body temperature of the animals.

Conclusion: The mechanisms that participate in the mediation of the PACAP-38-induced hyperthermia may be modified by isatin. The capability of isatin to antagonize the hyperthermia induced by all members of the natriuretic peptide family and by PACAP-38 makes it unlikely to be acting directly on receptors for natriuretic peptides or on those for PACAP in these hyperthermic processes.

Background

Pituitary adenylate cyclase-activating polypeptide (PACAP) is known as a member of a superfamily that includes vasoactive intestinal polypeptide (VIP), secretin, glucagon, gastric inhibitory polypeptide and growth hormone-releasing factor [9]. The biologically active neuropeptide exists in two amidated forms: PACAP-38, a 38-amino-acid polypeptide; and PACAP-27, a truncated form of PACAP-38 containing 27 residues [4]. Both PACAP-38 and PACAP-27 are potent in stimulating adenylate cyclase.

At least two receptor classes have been reported for PACAP in mammalian tissues: type I and type II. Type I receptors are highly selective in the recognition of PACAP, and have

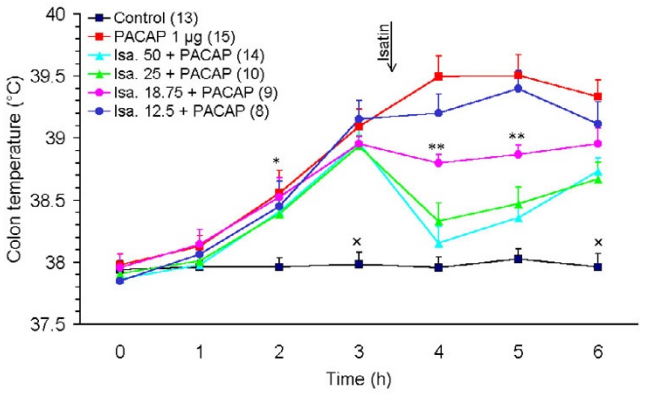

Figure 1
Effects of isatin on PACAP-38-induced hyperthermia. Groups of rats received an i.p. injection of saline (PACAP 1 µg group) or different doses (12.5, 18.75, 25 or 50 mg/kg) of isatin (Isa. + PACAP groups) 3.5 h after an i.c.v. injection of 1 µg PACAP-38. The control group received an i.p. saline injection 3.5 h after i.c.v. saline treatment (groups of animals receiving different dilutions (12.5, 18.75, 25 or 50 mg/kg; i.p.) of isatin 3.5 h after an i.c.v. saline injection are not shown). Number of animals per group is presented in parentheses after the corresponding group. The vertical lines on the top of the marks denote the S.E.M. The vertical arrow denotes the time of isatin injection (3.5 h). * $p < 0.05$ PACAP vs. other groups; × $p < 0.05$ control vs. other groups; ** $p < 0.05$ Isa. 18.75 + PACAP compared with the control, the Isa. 25 + PACAP, the Isa. 50 + PACAP and with the PACAP 1 µg groups.

lower affinity for VIP. Type II receptors display similar high affinity for PACAP-27, PACAP-38 and VIP [2]. Type II (VIP-PACAP) receptors interact almost exclusively with adenylate cyclase. At least two effector systems exist for type I PACAP-preferring receptors, which can stimulate both adenylate cyclase and phospholipase C. Subsequent to phospholipase C activation via the inositol phosphate cascade, a secondary Ca^{2+} entry elevating cytosolic [Ca^{2+}] follows Ca^{2+} mobilization [9].

Recent investigations carried out in our laboratory demonstrated that PACAP-38 [5] induced dose-dependent elevations in the body temperature of rats when injected centrally. Different members of the natriuretic peptide family had a similar effect [7]. The natriuretic peptides-induced hyperthermia was abolished by the endogenous indole isatin (indole-2, 3-dione) [6]. Isatin has been found to act as an antagonist at natriuretic peptide receptors of the brain and heart, and it may well be that isatin is an endogenous inhibitor of these peptides at a central level [3]. Although the antagonism by isatin of some of the behavioral effects of ANP has been described [1], much remains to be understood concerning the interaction of isatin and neuropeptide systems.

The present study was carried out to investigate the effects of isatin on PACAP-38- induced hyperthermia.

Results

Effects of isatin on the PACAP-38-induced hyperthermia

I.c.v. administration of PACAP-38 (1 µg) elevated the colon temperature 2, 3, 4, 5 and 6 h after administration. These observations were in accordance with our previously published data [5]. An I.p. injection of different doses of isatin (18.75, 25 and 50 mg/kg) 3.5 h after peptide treatment significantly diminished the hyperthermia in PACAP-38-treated animals at 4 and 5 h in a dose-dependent manner [$F(9, 97)_{2,3,4,5,6\,h} = 3.75, 24.26, 22.71, 24.91, 20.21$; $p < 0.05$]. Isatin had no antihyperthermic effect at 6 h (Fig. 1). Control injections of isatin (12.5, 18.75, 25 and 50 mg/kg; i.p.) 3.5 h after i.c.v. saline administration did not modify the colon temperature (data not shown).

Discussion

We have previously demonstrated that central administration of PACAP-38 produces a long lasting hyperthermia in rats, raising the possibility that this neuropeptide plays a part in thermoregulatory processes at a central level. No effect of the peptide on body temperature is seen 24 h after injection [5]. Other results provided evidence that the endogenous indole isatin inhibited the hyperthermia induced by natriuretic peptides [6].

The effects of isatin on PACAP-38-induced hyperthermia were investigated in this study. Injections of PACAP-38 into the lateral ventricle of the brain elevated the body temperature of the experimental animals with an onset of the hyperthermia at 2 h and a decline at 6 h. Single injections of isatin doses after the onset of the peptide-induced hyperthermia diminished the hyperthermic effect of PACAP-38 whereas isatin itself did not modify the body temperature. A positive correlation was observed between the antihyperthermic effect and the dose of isatin administered. No effect of the indole on hyperthermia was shown 2.5 h after injection.

An unpublished pilot study in our laboratory indicated that isatin did not have noticeable effect on the hyperthermia induced by PACAP-38 when injected before the onset of the body temperature elevation. This accounted for the isatin treatment 3.5 h after PACAP injection but does not support a possible interaction between PACAP receptors and the indole in the antihyperthermic effect of isatin.

The present results demonstrate that isatin is able to diminish the hyperthermic effect of neuropeptides other than natriuretic peptides, namely that of PACAP-38. The capability of isatin to antagonize the hyperthermia induced by all members of the natriuretic peptide family and also by PACAP-38 raises the possibility of common

pathways in the mediation of these neuropeptide-induced hyperthermic actions. However the hyperthermias induced by natriuretic peptides display earlier onset and shorter duration than that induced by PACAP-38. This makes it unlikely that isatin acts directly on receptors of natriuretic peptides or on those of PACAP in these hyperthermic processes. Moreover it does not support a possible action of the indole on an uncharacterized target that links the actions of natriuretic peptides and PACAP-38 to each other. To our knowledge no data are yet available on central actions of PACAP and natriuretic peptides being interrelated.

The exact mediatory pathway(s) of the PACAP-38-induced hyperthermia has not been clarified. However the involvement of prostaglandin products and possibly interleukins in the mediation are possible [5]. On the basis of our present data the connections of isatin to the interleukin-prostaglandin pathway in hyperthermic events and particularly in the hyperthermic effect of PACAP-38 require further examination.

Our data indicate that the hyperthermic effect of centrally administered PACAP-38 is inhibited by isatin in rats and establishes an *in vivo* interaction between isatin and PACAP.

Materials and Methods

Animals

The animals were kept and treated under a protocol accepted by the Committee on Animal Experiments, University of Szeged, Hungary.

Adult male Wistar rats weighing 200-240 g were used. Each animal was used only once for the experiments. The animals were housed in groups of 5-6 in cages in a room maintained at constant temperature (23 ± 1 °C) and on a standard illumination (lights on from 6 to 18). They had free access to tap water and standard laboratory food. Rats were allowed a minimum of 5 days of recovery from surgery before the beginning of experiments.

Surgery

In order to allow intracerebroventricular (i.c.v.) peptide administration, the rats were implanted with a unilateral cannula introduced into the right lateral brain ventricle before the experiment. Under pentobarbital (Nembutal, Ceva, France; 35 mg/kg) intraperitoneal (i.p.) anesthesia, the stainless steel cannula was inserted stereotaxically into the ventricle with coordinates 0.2 mm posterior; 1.7 mm lateral to the bregma; 3.7 mm deep from the dural surface, according to the atlas of Pellegrino *et al.* (1979). The cannula was secured with dental acrylic cement. Rats were allowed a minimum of 5 days to recover from surgery before peptide treatment. Upon the conclusion of the experiments, 10 µl methylene blue was injected into the ventricle of the decapitated animals and the position of the cannula was inspected visually. Animals with improper cannula placement were excluded from the final statistical analysis.

Treatments

Rat PACAP-38 was purchased from Bachem (Switzerland) or was synthesized by G. Tóth. For i.c.v. treatment, different doses of PACAP-38 were dissolved in sterile pyrogen-free 0.9% saline and injected in a volume of 2 µl. Isatin was administered i.p. in doses of 12.5, 18.75, 25 and 50 mg/kg, diluted in saline in a volume of 2.5 ml/kg. The control groups were treated with saline for all experiments.

Procedures

On the day of the experiment, the animals were transferred to the laboratory 2 h before the beginning of the test in order for them to habituate to the experimental environment. The room temperature was maintained at 23.0 ± 0.5°C throughout the experiment. Each animal was then removed from the cage and gently restrained with a cloth on the table. The colon temperature was monitored by inserting the vaseline-lubricated thermistor probe of a digital electric thermometer (Model: Cole-Parmer 8402-10) 5 cm into the rectum of the animal. The probe was inserted the same distance into the rectum each time it was used.

The experiments started at 8 a.m. with an initial colon temperature measurement, the colon temperature also being measured before and 1, 2, 3, 4, 5 and 6 h after peptide treatment.

The effects of i.p. administered isatin on the PACAP-38-induced hyperthermia were studied. Different doses (12.5, 18.75, 25, 50 mg/kg) of isatin were given i.p. once 3.5 h after i.c.v. peptide treatment (PACAP-38 being injected in a dose of 1 µg) and the colon temperature was measured 1, 2, 3, 4, 5 and 6 h later.

Statistical analysis

Statistical analysis of the data was made by analysis of variance (ANOVA). For significant ANOVA values, groups were compared by Tukey's test for multiple comparisons with unequal cell size. A probability level of 0.05 was accepted as indicating significant differences.

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