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Antipyretic effect of central [Pyr^1]apelin13 on LPS-induced fever in the rat

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ABSTRACT

Intracerebroventricular (i.c.v.) injections of apelins have been shown to modulate the central control of cardiovascular function, as well as the homeostasis of fluid and salt balance, and to some extent also body core temperature. Here, we investigated the effects of i.c.v. administration of [Pyr^1]apelin13 (PyrAp13; 20 nmol) dissolved in artificial cerebrospinal fluid (aCSF), as compared to aCSF alone, on fever and sickness behavior elicited in rats by intraperitoneal injection of bacterial lipopolysaccharide (LPS, 100 μg/kg). Injections of LPS induced a short phase of hypothermia followed by a biphasic fever, depression of motor activity, anorexia and adipsia. I.c.v. injections of PyrAp13 without systemic LPS application slightly augmented motor activity at statistically unaltered core temperature. In combination with LPS, central administration of PyrAp13 significantly reduced fever during the time period of 3–9 h after injection, but did not significantly attenuate anorexia and adipsia, and had no effect on LPS-induced lethargy. Rats injected with PyrAp13 along with LPS showed a reduced level of LPS-induced circulating tumor necrosis factor-α (TNF-α). Primary neuroglial cultures established from the hypothalamic paraventricular nucleus (PVN) and the median preoptic nucleus (MnPO), brain sites being of major importance for central thermoregulation and also expressing the apelin receptor, were incubated with medium alone, medium containing LPS (100 μg/ml) or LPS plus PyrAp13 (10^{-6} mol/L). Ninety minutes after start of the incubation, LPS alone but not LPS in combination with PyrAp13 (10^{-6} mol/L) caused a significant elevation of TNF-α in the supernatants. The novel observation that PyrAp13 represents a centrally acting endogenous antipyretic peptide is discussed in relation to its capacity to modulate peripheral and central formation of TNF-α.

1. Introduction

The G-protein coupled apelin receptor APJ sharing a 31% sequence homology to the angiotensin II (AngII) receptor subtype 1A was discovered in 1993 [1]. Apelins, the peptidergic ligands of APJ, are synthesized as a 77 amino acid precursor with the sequence of the last 23 amino acids being conserved amongst all mammals examined, and are subsequently cleaved C-terminally into the bioactive peptides apelin13, apelin17 or apelin36. The post-translationally modified N-terminal pyroglutamyl [Pyr^1] form of apelin13 (PyrAp13) preserves biological activity, is protected against rapid enzymatic degradation and represents the predominant apelin peptide detectable in brain tissue [2]. All apelin peptides show high binding affinity to APJ and have been demonstrated to be involved in a variety of vital homeostatic and autonomic processes. The apelinergic system has a strong influence on the cardiovascular system, participates in the control of energy metabolism and plays a role in the homeostasis of salt and water balance [3–6]. In line with these observations, dense expression of apelins and its receptor APJ have been demonstrated in hypothalamic nuclei involved in fluid and salt balance regulation, including the paraventricular nucleus (PVN) and the median preoptic nucleus (MnPO) [6–9]. Both of these structures not only play a role in the central control of water balance, but also of thermoregulation and fever [10–13]. There exist, however, only a few published studies dealing with effects of intracerebroventricular (i.c.v) administration of apelins on body temperature [14,15]. Both studies indicated that acutely [14] or chronically [15] i.c.v. administered apelin13 exerted a temporary elevation of body core temperature in rats or mice, which was associated with a higher state of exploratory (rats) or telemetrically recorded (mice) locomotor activity, the latter underlying the slight hyperthermic response to central apelin in mice. Up to now, no studies have been performed on a putative influence of apelin on lipopolysaccharide (LPS)-induced fever, which is the most frequently used model of experimental fever [16]. This question is of interest in so far as there are a number of neuropeptides, which have the capacity to act as central modulators of the febrile response. Some brain-intrinsic neuropeptides including endothelin-1 [17,18], corticotrophin-releasing factor (CRF) [18,19] or AngII [20] have fever-promoting effects, while others such as [Arg^8]vasopressin or α- and γ-melanocyte-stimulating hormones (α-MSH and γ-MSH) possess capacities as endogenous antipyretics [11,21–24].

Abbreviations: aCSF, artificial cerebrospinal fluid; AngII, angiotensin II; APJ, apelin receptor; CRF, corticotrophin-releasing factor; h, hour(s); HPA axis, hypothalamo-pituitary-adrenal axis; i.c.v., intracerebroventricular; I.kU., kilo unit = 1000 U; IL-6, interleukin-6; i.p., intraperitoneal; LPS, lipopolysaccharide; MnPO, median preoptic nucleus; PVN, paraventricular nucleus; PyrAp13, [Pyr^1]apelin13; TNF-α, tumor necrosis factor alpha.

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Therefore, the central goal of this study was to assess a possible influence of i.c.v. administered PyrAp13 on LPS-fever, lethargy, anorexia and adipsia as well as pro-inflammatory cytokines, components of the sickness behavior in rats. We confirm that PyrAp13 *per se* tends to augment locomotor activity. We further demonstrate for the first time that centrally injected PyrAp13 exerts antipyretic effects, but not accompanied by reduced motor activity, anorexia and adipsia in LPS treated rats. The antipyretic capacities of apelin13 analogues are discussed in the context of their inhibitory influence on LPS-induced endogenous formation of tumor necrosis factor-α (TNF-α).

2. Materials and methods

2.1. Animals

Male Wistar rats with an initial body weight in the range of 200–240 g, obtained from an in-house breeding colony, were used for this study. All experiments were carried out in accordance with the local ethics committee (approval number: v54-19 c20/15 (1) 18/2 Nr. 66/2011). After surgery (see Section 2.3), animals were housed for recovery in normal standard cages under a 12:12 h day–night cycle with lights on from 7:00 a.m. to 7:00 p.m. and *ad libitum* access to standard rodent food (Altromin, Lange, Germany) and drinking water. Four days after surgery, animals were placed individually in special custom made cages in a temperature and humidity controlled climatic chamber (Typ10′US/+5 to +40 DU; Weiss Umwelttechnik, Reiskirchen, Germany) at 25 °C and 50% humidity, again under a 12:12 h day–night cycle with lights on from 7:00 a.m. to 7:00 p.m.

To continuously measure the intake of food and water, food supply dishes with powdered standard chow food (Altromin, Lange, Germany) and the water bottles were both placed on balances, the output of which being fed *via* a DietScan analyzer (AccuScan Instruments, Columbus, OH, USA) to a personal computer. Body weight was determined once a day and animals were handled and trained for i.c.v. injections to reduce stress during the acute experiments.

2.2. Substances

PyrAp13 (Bachem, Bubendorf, Switzerland) was dissolved in sterile artificial cerebrospinal fluid (aCSF) (in mmol/L): NaCl 125, KCl 2.5, NaH_2PO_4 1.25, $NaHCO_3$ 26, $CaCl_2$ 2, $MgCl_2$ 1, and D-glucose 25, with all chemicals purchased from Sigma-Aldrich (Deisenhofen, Germany). A dose of 20 nmol PyrAp13 in a total volume of 2 μl aCSF was used for i.c.v. injection. This dose was selected according to other studies performed with rats, in which i.c.v. injections of 1–20 nmol PyrAp13 or apelin13 had significant cardiovascular effects and induced neuronal activation in the hypothalamic PVN [25], or caused an activation of the hypothalamo-pituitary–adrenal (HPA) axis and a mild rise in core temperature [14].

LPS derived from *Escherichia coli* (serotype O111:B4; Sigma Aldrich) was suspended in sterile phosphate-buffered saline (PBS) at a concentration of 100 μg/ml. A dose of 100 μg/kg LPS was used for intraperitoneal (i.p.) injections. This dose has been shown to induce fever and sickness behavior in rats when injected *via* the i.p. route [26,27].

2.3. Surgery

One hour prior to surgery, each rat was injected with the analgesic Metacam® (2.25 mg/kg; Boehringer, Ingelheim, Germany) into the intrascalpular subcutaneous tissue. The animal was deeply anesthetized by i.p. injection of a mixture of Cepetor® (330 μg/kg; cp-Pharma, Burgdorf, Germany) and ketamine hydrochloride (80 mg/kg; Bela Pharm, Vechta, Germany). A biotelemetry transmitter (VM-FH Discs; Minimitter, Bend, OR, USA) was implanted into the abdominal cavity for later measurements of abdominal body temperature and motor activity. The rat was then transferred and placed into a stereotaxic frame (M-900; David Kopf Instr., Tujunga, CA, USA) and an incision was made in the midline of the cranium to expose the skull. A 23-gauge stainless steel guide cannula (Plastics One, Roanoke, VA, USA) was introduced into the brain through a pre-formed skull trepanation over the right lateral cerebral ventricle (stereotaxic coordinates: 0.8 mm posterior to bregma, 1.5 mm lateral to the midline and 2.5 mm ventral from the skull surface) [28]. The guide cannula was fixed to the skull and four implanted stainless steel screws with dental cement (Stoelting Europe, Dublin, Ireland). A sterile dummy cannula with a lid (Plastics One) was used for transient obturation of the cannula system. To antagonize anesthesia, the α_2-antagonist Antisedan® (1.13 mg/kg; Pfizer, Berlin, Germany) was given intramuscularly. During the process of surgery and also during recovery from anesthesia, the animal was kept on a heating pad (departmental workshop) to avoid hypothermia. After surgery, body weight, behavior and the wound healing process were controlled daily, and 24 h after the first analgesia animals were given a second injection of Metacam® (1.13 mg/kg).

To prove correct placement of the cannula in the lateral ventricle, an established drinking test [29] was performed. AngII (50 pmol; Bachem) dissolved in 2 μl sterile aCSF was slowly injected (30 s) into the lateral ventricle using an internal injection cannula (Plastics One), which protruded 2 mm beyond the tip of the guide cannula. The drinking response was measured within 30 min after injection, and only animals which drank at least 9 ml within that time were used for the experiments [29]. The drinking test was conducted 7–8 days after i.c.v. cannulation and 3–4 days before experimental stimulation (see below).

2.4. Telemetric recording of body temperature, locomotor activity, food and water intake

Body core temperature, motor activity, food and water intake were recorded every 5 min by telemetric device systems for body temperature and activity (Vital View, Mini Mitter), and for food and water intake (AccuScan) as described previously [27,30]. Food and water intake as well as activity data were evaluated and are shown as delta percentage (Δ%) of cumulative basal values for selected time intervals. For basal values, means of the parameters of each animal were calculated from data obtained for the same time span of days one and two prior to the injection day. Body core temperature data were calculated and are shown as delta values (ΔTb) in relation to basal temperature data, which were calculated as daily means of the two days preceding the injection day.

2.5. Neuroglial primary cell cultures from the PVN and MnPO

Primary microcultures of the rat PVN and MnPO were established from topographically excised brain tissue fragments obtained from 4–6 days old Wistar rat pups. To obtain primary cell cultures, animals were decapitated and brains quickly removed under aseptic conditions and transferred to small Petri dishes filled with ice-cold oxygenated Gey's Balanced Salt Solution (GBSS; Biotrend, Köln, Germany) containing 5% D-glucose (Sigma-Aldrich). Brains were positioned in a vibratom (725 M Vibroslice; WPI, Berlin, Germany) filled with ice-cold, oxygenated GBSS, and serial coronal 400 μm slices were obtained from the hypothalamic region. Using the anterior commissure, the optic chiasm and the 3rd cerebral ventricle as neuroanatomical landmarks [28], the PVN and MnPO were microdissected under stereomicroscopic control (SMZ-U; Nikon, Düsseldorf, Germany) using fine eye scissors. To check for correct excision of the respective hypothalamic sites, their neuroanatomical location was compared to that in cresyl violet stained neonatal rat brain slices as well as coronal brain sections immunohistochemically marked for [Arg^8]vasopressin and neuronal nitric oxide synthase [9]. Isolated PVN and MnPO tissue fragments of 5–6 rat pups were collected in Petri dishes containing ice-cold oxygenated Hank's Balanced Salt Solution (HBSS) without Ca^{2+} and Mg^{2+} (Biochrom, Berlin, Germany) and supplemented with 20 mmol/L HEPES (Sigma-Aldrich), pH 7.4. After removal of the supernatant, both preparations were then treated with