Acidic fibroblast growth factor activates hypothalamic-pituitary-adrenocortical axis in rats

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Matsumoto, Itsuo, Yutaka Oomura, Akira Niijima, Kazuo Sasaki, and Tadaomi Aikawa. Acidic fibroblast growth factor activates hypothalamic-pituitary-adrenocortical axis in rats. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R503–R509, 1998.—Effects of acidic fibroblast growth factor (aFGF), an endogenous satiety substance, on the hypothalamic-pituitary-adrenocortical axis were examined under pentobarbital sodium anesthesia in rats. A guide cannula was inserted into the cerebral third ventricle and a vascular indwelling catheter was inserted into the right atrium from the jugular vein 2 wk and 3 days, respectively, before the experiment. A marked dose-dependent increase in plasma corticosterone was detected from 20 min to 2 h after intracerebroventricular administration of aFGF (1–10 ng). Significant increases in plasma adrenocorticotropic hormone (ACTH) were observed from 5 to 150 min after the intracerebroventricular administration of 10 ng aFGF. Significant dose-dependent increases in plasma corticosterone were also observed after intravenous injections of aFGF (1, 10, and 100 ng), together with increases in the plasma ACTH level. Pretreatment with antibody to corticotropin-releasing factor via the intracerebroventricular route abolished the increases in corticosterone induced by intracerebroventricularly administered aFGF, but not those induced by intravenous injection of aFGF. In adrenal glands perfused in situ with artificial medium, the corticosterone secretion rate increased slightly in response to 10−8 M aFGF. These findings suggest that intracerebroventricular administration of aFGF activates the hypothalamic-pituitary-adrenal axis via corticotropin-releasing factor release in the brain, whereas peripheral administration of aFGF activates adrenocortical secretion mainly via a direct action on ACTH release.

CORTICOTROPIN-RELEASING factor (CRF), a 41-amino acid peptide, is widely distributed throughout the spinal cord and brain, particularly the hypothalamus (27). CRF serves to regulate the activities of the hypothalamic-pituitary-adrenal (HPA) axis and, thus, for example, the behavioral and autonomic responses to stress. CRF is closely associated with anorexia in humans (16) and with suppression of food intake in rats (2). Microinjection of CRF into the ventromedial hypothalamus (VMH) diminishes the gastric mucosal damage induced by cold restraint (12). Furthermore, in Alzheimer’s disease, reciprocal changes in CRF-like immunoreactivity and CRF receptors have been observed in the cerebral cortex (4). Such a demonstration of an upregulation of CRF receptors after a decrease in CRF-like immunoreactivity suggests a contribution of CRF to cognitive processes (4, 7, 8).

Acidic (a) and basic (b) fibroblast growth factors (FGFs) belong to the family of heparin-binding polypeptide growth factors that influences the proliferation and differentiation of various cell types in vitro. aFGF exhibits ~55% sequence identity with the basic type, and both interact with the same cell surface receptors (24). FGFs are produced by ependymal cells located in the third cerebral ventricle (26), and they are released into the cerebrospinal fluid after feeding or intraperitoneal injection of glucose in rats (13). Infusion of FGFs into the third cerebral ventricle dose dependently inhibits food intake (26, 29), the aFGF-induced effects being about twofold stronger than those of bFGF (26, 29). Moreover, infusion of antibodies against aFGF into the lateral hypothalamic area (LHA) provokes food intake (31). Electrophoretic application of aFGF suppresses the activity of glucose-sensitive neurons in the LHA (13) through the activation of protein kinase C (26). In addition, Oomura et al. (26) have reported that endogenous aFGF is involved in learning and memory processes in rats. It is evident that aFGF-induced responses are similar to those obtained on administration of CRF or 2-buten-4-olide (2-B4O), one of the endogenous satiety substances (1, 25, 26). These data suggest the possibility that aFGF induces the release of CRF in the brain and that the CRF thus released promotes a variety of in vivo physiological reactions in the same way as 2-B4O (22). The purpose of the present study was to investigate whether, and how, aFGF activates the HPA axis in rats.

MATERIALS AND METHODS

Animals. Male Wistar rats (300–350 g) were housed individually in a room with temperature maintained at 24 ± 1°C and in a 12:12-h light-dark cycle (lights on at 0700). All rats had free access to food and water. The protocols conformed with guidelines on the conduct of animal experiments issued by the Animal Care and Use Committee of our university and by the Japanese government (Law No. 105, Oct. 1, 1973).

Surgical procedures. In each rat, a guidencannula (stainless steel tubing, 23 gauge) was fixed into the third cerebral ventricle 2 wk before the experiment through a hole drilled into the skull. Three days before the experiment, a silicon catheter (OD 1.0 mm) was inserted into the right atrium through the right jugular vein and brought out subcutaneously at the back of the neck. Through the implanted catheter, 0.4-ml blood samples were slowly drawn, as required, over a period of 2 min. The blood cells from each sample were resuspended in the same volume of saline and returned to the...
animal before the next sampling period. Such replacements ensured that the hematocrit of all blood samples remained between 42 and 50%. Plasma was stored at −80°C with 0.2% EDTA for not more than 4 wk until assayed for adrenocorticotropic hormone (ACTH) and corticosterone. All experiments involving blood sampling were performed between 0900 and 1400. Three weeks before the experiment, the splanchic nerves were sectioned bilaterally below the diaphragm in nine animals, and the hepatic branch of the left vagus nerve was dissected free and sectioned in eight rats. All procedures were performed under pentobarbital sodium anesthesia (50 mg/kg).

Bilateral perfusion of adrenal glands. The method employed for the in situ perfusion was similar to one described elsewhere (36). In brief, the abdomen was opened by a midline incision from the xiphoid process to the pubic symphysis. The incision was extended laterally into right and left flanks by subcostal incisions. For access to the bilateral adrenal glands, the viscera were removed after ligation of the celiac artery, superior and inferior mesenteric arteries, and portal vein. The abdominal aorta and vena cava were ligated above the exit of the inferior mesenteric artery. The renal artery and vein and spermatic vein were ligated. This procedure left intact the vessels supplying and draining the adrenal glands. Silken threads were loosely placed around the abdominal aorta below the diaphragm and the vena cava just below the hepatic vein. A polyethylene cannula (OD 1.35 mm) directed toward the heart was inserted into the abdominal aorta below the renal artery, and then the dorsal aorta was ligated immediately below the exit of the celiac artery. A cannula was placed in the vena cava so as to allow collection of perfusate from the adrenal glands. The silk threads were tightened after the start of the perfusion. The inflow catheter, which was covered with a water jacket maintained at 38°C, was connected to a peristaltic minipump (ATTO). This pump was adjusted to deliver the perfusion medium at between 400 and 500 µl/min. Preliminary perfusion for 60 min with Krebs-Ringer bicarbonate glucose solution (KRBG) containing 1% bovine serum albumin allowed the adrenal glands to reach a steady state before the experimental manipulations were started. The secretion rate for corticosterone (ng·min⁻¹·100 mg adrenal wt⁻¹) was calculated from the concentration of corticosterone in the perfusate (ng/ml) and the flow rate of the medium perfusing the adrenal glands (ml·min⁻¹·100 mg adrenal wt⁻¹). When the perfusate contained aFGF or ACTH, the period of perfusion was 5 min.

Administration of drugs. aFGF (R & D Systems) was dissolved in 10 µl artificial cerebrospinal fluid (CSF) containing 0.1% bovine serum albumin and administered over 2 min via the implanted intravenous catheter or via an intracerebroventricular needle (29 gauge) fixed into the cerebral guide cannula. Lyophilized anti-CRF antibody (contained in 50 µl rabbit antiserum against human CRF; Peptide Institute) was dissolved in 50 µl artificial CSF, and 10 µl of this anti-CRF antibody solution containing 10 µl antiserum was given intracerebroventricularly to 10 animals 20 min before the start of the experiment.

Measurements. The concentration of ACTH in plasma was determined using commercially available radioimmunoassay kits (CIS Biointernational). In brief, the anti-ACTH antiserum used in this assay was produced polyclonally by rabbits using human ACTH for antigen, and it reacted to the 1–24 amino acid residues of ACTH. It showed about 85% cross-reaction to artificially synthesized ACTH-(1–24) and ~80% to rat ACTH at a mean value of 50 pg/tube, but it showed no cross-reaction to α- or β-melanocyte-stimulating hormone. Each plasma sample (0.1 ml) was assayed in duplicate. The sensitivity of the assay was 2 pg/tube. The inter- and intra-assay coefficients of variation at a mean value of 50 pg/tube were 12.3 and 10.6%, respectively. Plasma corticosterone was measured by the previously described method (22). We also calculated the integrated response (area under the curve) for the increase in corticosterone in the plasma or perfusate induced by aFGF or ACTH and for the increase in plasma ACTH induced by aFGF. These integrated responses were expressed as the incremental increase above the respective basal concentrations over a period of 180 min (for plasma levels) or 60 min (perfusate levels) after administration of the agent.

Statistical analysis. Data were analyzed by one-way or two-way analysis of variance (ANOVA), with a correction for repeated measures, by means of a computer software program for statistical analysis, as previously reported (22). When a significant overall effect was revealed by ANOVA, the significance of differences from baseline within a given group and between groups at each time point was tested by appropriate post hoc statistics using the same computer software system.

RESULTS

Effects of aFGF administered intracerebroventricu-
larly on plasma corticosterone. Significant increases in plasma corticosterone concentration were evoked in a dose-dependent manner in response to intracerebroventricularly administered aFGF (at 1 and 10 ng; Fig. 1). The corticosterone levels reached a maximal level at 60 min and remained elevated for up to a further 120 min after the administration. The integrated corticosterone responses (for the 180 min after the administration) also showed a dose-dependent increase (Fig. 1, inset).

Effects of aFGF administered intravenously on plasma corticosterone. Significant increases in plasma corticosterone were evoked in a dose-dependent manner in response to intravenously administered aFGF (at 1, 10, and 100 ng; Fig. 2). The corticosterone levels peaked at 60 min and then tended to gradually decrease toward the basal level over the remainder of the 180-min period after the administration. The integrated corticosterone responses also increased in a dose-dependent manner (Fig. 2, inset). The responses to aFGF adminis-
Effects of aFGF administered intracerebroventricularly or intravenously on plasma ACTH. Changes in the plasma levels of ACTH after intracerebroventricular or intravenous administration of 10 ng aFGF are shown in Fig. 3. When 10 ng aFGF was administered via the intracerebroventricular route, the ACTH concentration was already increased significantly at 5 min after the injection; it continued to increase and peaked at 15 min after the injection. Thereafter, it gradually decreased, but remained elevated until 150 min after the injection; it had returned to the basal level at 180 min. At this time (180 min), the plasma corticosterone was still at an elevated plateau level after its peak at 60 min (see Fig. 1). A significant increase in ACTH concentration was also evoked by intravenously administered aFGF (10 ng). The ACTH level increased significantly and peaked at 15 min after the injection, then rapidly decreased; it had returned to the basal level 90 min after the injection. As can be seen in Fig. 2, the plasma corticosterone level had not yet returned to the basal level at this time. The ACTH level seen after the intracerebroventricular administration of 10 ng aFGF was significantly greater than that seen after the intravenous administration of the same dose at 5, 10, 90, 120, and 150 min after the injection. The integrated ACTH response evoked by intracerebroventricular aFGF was about twofold greater than that evoked by intravenous aFGF (Fig. 3, inset).

Effect of anti-CRF antibody. The effects of pretreatment with anti-CRF antibody on the increases in plasma corticosterone induced by intracerebroventricular and intravenous administrations of 10 ng aFGF are shown in Fig. 4. In both cases, the basal level of corticosterone in rats that had received the pretreatment was not significantly different from that in rats without pretreatment. The pretreatment significantly attenuated the increase in corticosterone evoked by intracerebroventricular administration of aFGF, but had almost no effect on the response to its intravenous administration. The integrated corticosterone responses clearly showed these effects of pretreatment with anti-CRF antibody (Fig. 4, inset).
Direct effect of aFGF on the adrenal cortex. Changes in the rate of corticosterone secretion from adrenal glands perfused with KRBG in situ were evoked by aFGF and by ACTH (Fig. 5). Corticosterone secretion exhibited significant biphasic increases during perfusion with either 1 or 10 nM aFGF, the peaks occurring at -5 and 50 min after the start of the perfusion. The integrated corticosterone responses to 1 and 10 nM aFGF and to 30 (7 pM) and 100 pg/ml (22 pM) ACTH (measured over 60 min) are shown in Fig. 5, inset. The responses to both agents were dose dependent. Although the integrated responses to aFGF were significantly greater than those to the vehicle control, they were significantly smaller than those induced by ACTH.

Effects of splanchnicotomy and hepatic vagotomy on the increases in corticosterone. It is well known that catecholamines induce ACTH secretion from the pituitary. Because we found that aFGF administration via either route evoked not only a marked increase in the plasma level of epinephrine, but also significant increases in norepinephrine (data not shown), the effects of bilateral splanchnicotomy (SPX) were examined on the increases in corticosterone induced by 10 ng aFGF given intravenously or intracerebroventricularly (Fig. 6). The significant increase in the integrated corticosterone response was still present after SPX (Fig. 6), but there was no increase in epinephrine (data not shown). In SPX rats, the temporal pattern of the increase in plasma corticosterone evoked by aFGF was almost the same as that seen in intact animals. In other experiments, hepatic vagotomy (HVX) also failed to alter the increases in plasma corticosterone (Fig. 6), although an increase in epinephrine was not evoked after HVX (data not shown).

DISCUSSION

In this study, the plasma level of corticosterone increased in a dose-dependent fashion after the intracerebroventricular or intravenous administration of aFGF. However, the integrated corticosterone response at the same aFGF concentration was 1.4–2 times higher when aFGF was given intracerebroventricularly than when it was given intravenously. The increase induced by intracerebroventricular aFGF was severely attenuated by pretreatment with anti-CRF antiserum via the intracerebroventricular route, but the intravenous response was not affected at all. This suggests that aFGF causes corticosterone release via CRF release in the brain when administered intracerebroventricularly, but via a direct action on ACTH release from the pituitary gland when given intravenously (see below). Recently, we found that microelectrophoretic application of aFGF to the parvocellular neurons of the paraventricular nucleus (PVN) in vitro facilitated neuronal activity in more than one-third of the neurons tested (32). These pieces of evidence suggest that endogenous aFGF or exogenous aFGF (given intracerebroventricularly) directly stimulates CRF secretion from parvocellular neurons and that this CRF then activates the pituitary-adrenal axis.

When injected intravenously, aFGF increased the plasma level of ACTH, as well as corticosterone levels. Unexpectedly, and as mentioned above, pretreatment with anti-CRF antiserum via the intracerebroventricular route did not attenuate the corticosterone increase evoked by intravenous aFGF. The anterior pituitary is known to be rich in FGFs (10) and to have FGF receptor 1 at a high density (11). Receptor 1 for FGFs is also found in the adrenal cortex in rats (A. Kinoshita, I. Tooyama, Y. Oomura, I. Akiguchi, and H. Kimura, unpublished observation). In addition, FGFs potentiate the secretion of thyrotropin and prolactin from the pituitary gland (3). These data indicate...
that aFGF injected via the intravenous route could act directly not only on the pituitary gland, but also on the adrenal cortex. Although the integrated adrenal secretory responses increased dose dependently after infusions of 1 and 10 nM aFGF into adrenal glands perfused with artificial medium, the responses were smaller than that induced by 100 pg/ml (22 PM) ACTH (although significantly greater than that to vehicle control). Furthermore, the integrated corticosterone responses were unaffected by SPX or by HVX, although each maneuver completely prevented the increase in epinephrine induced by aFGF in intact rats. Thus the main site of action of aFGF when given intravenously seems likely to be in the pituitary gland. However, it remains possible that unknown humoral factors liberated from the various peripheral organs after intravenous injection of aFGF might be involved in the activation of the pituitary-adrenal axis. Indeed, it has been shown that bFGF elicits a marked secretion of interferon-γ from natural killer cells without cellular proliferation in mice (18, 21), and that aFGF, but not bFGF, induces non-REM sleep and feeders of >1°C in rabbits (15, 20).

The aFGF concentration in the cerebrospinal fluid (CSF) may be within the physiological range after an intracerebroventricular administration of 10 ng (0.7 × 10^-12 mol) aFGF. In fact, if we assume that the total volume of the rat CSF is ~300 µl, the concentration of aFGF in the CSF would be ~2 pmol/ml (34). This would seem to lie within the normal range, because the aFGF concentration in rat CSF increases from ~0.7 pmol/ml to 0.7 nmol/ml at 15 min, 7.5 nmol/ml at 45 min, and 4.6 pmol/ml at 3 h after 4 mM glucose application into the cerebral ventricle (26) and after food intake (13). The figure of 4 mM glucose corresponds to the glucose level in the CSF after food intake or intraperitoneal injection of 300 mg/kg glucose (13). However, it is still unclear how aFGF stored in the central nervous system could be activated and which pathways may be involved.

Activation of the HPA axis by exogenous aFGF applied intracerebroventricularly is consistent with the idea that food intake has a close functional relationship to the regulation of the HPA axis. In fact, Hanson and Dallman (14) concluded, on the basis of the following evidence, that food intake is one of the major regulators of adrenocortical activation. 1) Neuropeptide Y (NPY) is an endogenous feeding substance produced in the arcuate nucleus, and neurons containing NPY synapse on cells synthesizing CRF in the PVN. NPY stimulates both food consumption and the activity of the HPA axis. 2) Rhythms in the activity of the HPA axis follow rhythms in food consumption. 3) Restricted feeding regimens can shift the pattern normally exhibited by the rhythms in HPA axis activity, so that the peak in adrenal activity coincides with the start of food consumption. Interestingly, NPY increases within the PVN just before feeding and acts to induce feeding (6). However aFGF, as mentioned above, increases in the CSF after feeding and acts as an endogenous satiety substance to stop feeding (13). Furthermore, centrally administered aFGF results in increased firing of sympathetic nerve fibers innervating brown adipose tissue (A. Nii-jima, unpublished observation) and in increased thermogenesis (20), responses contrary to those induced by central NPY. The minimum concentration of aFGF required for such suppression of feeding is ~3.3 pmol/ml. Both exogenous NPY and aFGF, when applied intracerebroventricularly, activate the HPA axis via release of CRF. However, because exogenous application of aFGF at 0.7 pmol (10 ng) and of NPY at 0.6 nmol (2.5 µg) produced approximately the same level of corticosterone in the plasma (14), it seems that aFGF is able to activate the HPA axis at a much smaller physiological dose than is NPY. Recently, Erickson et al. (9) reported some surprising results: in NPY gene-knockout (−/−) mice, body weight, food consumption, and sensitivity to leptin, a peripheral signal for the amount of fat stored, remained at normal levels. In fact, such mice appear to be normal in every respect, except for a propensity for seizures. These data may indicate that other substances can take the place of NPY in regulating the body weight. It is well known that CRF is closely associated with anorexia (16) and with suppression of food intake (2, 35). Involuntary overfeeding induces spontaneous hypophagia after termination of the overfeeding regimen, accompanied by a stimulation of CRF gene expression in the PVN (33). This demonstrates that an important role of CRF may be to maintain a stable state of energy balance by suppression of food intake. Thus an elevation of CRF induced by aFGF in the brain may be linked to satiation.

It is well known that plasma corticosterone peaks just before feeding in the dark period and remains at a high level for 6–8 h during this period. Thus an elevation of plasma corticosterone levels induced by aFGF after feeding might exert a permissive effect on the consumption of fuel after overeating. In so doing, aFGF might cause hyperthermia (Ref. 20; I. Matsumoto, unpublished observation when aFGF was intravenously administered) in concert with epinephrine and sympathetic outflow, because aFGF also increases plasma catecholamine levels and sympathetic efferent activity to interscapular brown adipose tissue (A. Nii-jima and I. Matsumoto, unpublished observation).

Perspectives

The present data suggest that the effects of aFGF on behavior may be mediated, at least in part, by endogenously increased glucocorticoids. aFGF released after feeding and/or peripheral glucose administration facilitates learning and memory in rats and mice (26). For example, in rats, continuous infusion of aFGF into the lateral cerebral ventricles by an osmotic minipump increases latency in retention trials in passive avoidance tests throughout the infusion time (26). Facilitated learning and memory after glucose injection, as revealed by performance in a passive avoidance or water maze task, is almost abolished by pretreatment with anti-aFGF antibody by intracerebroventricular administration (26). aFGF also facilitates, in a dose-dependent fashion, long-term potentiation in the CA1 region of the rat hippocampus in vitro (30). The CA1
region is one of the important target sites for corticosteroids, and corticosterone can both modulate the long-term potentiation (28) and enhance the afterhyperpolarization (17) in CA1 neurons in hippocampal slices via hippocampal corticosterone receptors. Thus part of the effect of aFGF on behavior in vivo may be the result of a direct action of the induced corticosterone increase on the hippocampus. CRF also promotes learning and cognitive processes (4, 8), as do corticosteroids in the rat (5). In fact, recent clinical data suggest that CRF deficiencies can be detected in the brain in patients with neurodegenerative dementia (7). Consequently, these data could suggest that CRF also plays an important role in the wide spectrum of autonomic, hormonal, and behavioral changes that can be induced either by endogenous aFGF after feeding or by exogenous intracerebroventricularly administered aFGF.

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