Acidic fibroblast growth factor activates adrenomedullary secretion and sympathetic outflow in rats

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Matsumoto, Itsuro, Akira Niijima, Yutaka Oomura, Kazuo Sasaki, Katsuhiko Tsuchiya, and Tadaomi Aikawa. Acidic fibroblast growth factor activates adrenomedullary secretion and sympathetic outflow in rats. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1003–R1012, 1998.—Effects of exogenous acidic fibroblast growth factor (aFGF), which is increased in the brain by food intake, on the plasma levels of catecholamines and on sympathetic efferent outflow were examined in anesthetized 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. Plasma epinephrine (Epi) and norepinephrine (NE) increased markedly in a dose-dependent manner for up to 120 min after intracerebroventricular or intravenous administration of aFGF (6–667 fmol/rat). Concomitant increases occurred in the efferent activity in the sympathetic nerves supplying the adrenal, spleen, and interscapular brown adipose tissue after the above administrations of aFGF. Both intravenous and intracerebroventricular administration of 10 ng basic FGF (bFGF) also increased sympathetic adrenal efferent activity and plasma Epi and NE concentrations. However, the increases induced by 10 ng bFGF were smaller than those induced by 10 ng aFGF. Bilateral splanchnicotomy completely prevented the increases in Epi induced by intracerebroventricular or intravenous aFGF but had less effect on the increases in NE. Pretreatment with an antibody against corticotropin-releasing factor (CRF), given via the intracerebroventricular route, significantly attenuated the increases in Epi and NE evoked by intracerebroventricular or intravenous administration of aFGF. Hepatic vagotomy also greatly reduced the increases in both catecholamines and the increases in sympathetic efferent firing rates evoked by intravenous administration of aFGF. These findings indicate that 1) aFGF administered intracerebroventricularly activates adrenomedullary secretion and sympathetic outflow via CRF release and 2) aFGF injected intravenously also induces sympathoadrenomedullary activation via centrally released CRF. The idea is discussed that sympathetic activation induced either by endogenous aFGF after feeding or by exogenously administered aFGF may play roles both in energy expenditure after overeating and in the modulation of immune functions.

sympathetic activity; corticotropin-releasing factor; energy expenditure; overfeeding; autoimmune function

ACIDIC AND BASIC fibroblast growth factors (aFGF and bFGF), which influence the proliferation and differentiation of various cell types in vitro, were originally isolated as single chain proteins from neural tissue, including whole brain and hypothalamus (3). Recently, it has become clear from a number of studies that FGFs have a wide spectrum of effects on neurophysiological activities that are distinct from their mitogenic action within the central nervous system (CNS) in vivo. For instance, when administered into the brain, aFGF protects against the degeneration of hippocampal CA1 neurons that is induced by brain ischemia (28). Endogenous aFGF enhances learning and memory processes and facilitates long-term potentiation in hippocampal CA1 neurons (16, 25, 27). Endogenous aFGF and bFGF are released from the ependymal cells of the cerebral third ventricle into the cerebrospinal fluid (CSF) after food intake (8, 29) and act as feed suppressants via the inhibition of glucose-sensitive neurons in the lateral hypothalamic area (LHA) (8, 15, 25, 29). Furthermore, intracisternal injection of bFGF both suppresses secretion of gastric acid and reduces the severity of experimentally induced gastric mucosal lesions in rats (23). Interestingly, aFGF exhibits –55% sequence identity with the basic type and the two forms interact with the same cell-surface receptors (20). However, the suppressant action of aFGF on feeding is about twofold stronger than that of bFGF (8), whereas aFGF but not bFGF induces both thermogenesis, leading to a<1°C increase in body temperature, and non-rapid eye movement (REM) sleep (14).

It is well known that corticotropin-releasing factor (CRF) mediates activation of the hypothalamo-sympathoadrenomedullary axis (5) as well as of the hypothalamic-pituitary-adrenal axis and that CRF is closely associated with anorexia in humans (9) and rats (1). In addition, it has been shown that a microinjection of CRF into the ventromedial hypothalamic area (VMH) diminishes the gastric mucosal damage induced by cold restraint (7). Furthermore, by its central action, an acute application of CRF facilitates the process of memory formation (17). Moreover, glucocorticoid is linked to long-term potentiation in CA1 neurons in the hippocampus (11). These observations led us to hypothesize that FGFs exogenously administered may lead to activation of the hypothalamo-sympathoadrenomedullary system via a release of CRF in the brain. This idea is consistent with the fact that exogenous and endogenous aFGF both induce reactions similar to the integrated physiological responses induced by CRF. Indeed, we have reported that when administered centrally exogenous aFGF potentiates the hypothalamic-pituitary-adrenal axis via CRF release (18). Thus the purpose of
this study was to investigate whether and how 1) aFGF activates sympathetic efferent activity, 2) aFGF increases plasma catecholamines, and 3) CRF in the brain is linked to the increase in catecholamines.

MATERIALS AND METHODS

Animals and surgery. Male Wistar rats weighing 350–400 g, housed individually with a 12:12-h light-dark cycle, were maintained at a room temperature of 24 ± 1°C with free access to food and water. All procedures were approved by the Animal Care and Use Committee of Nagasaki University and conformed to guidelines for the use of laboratory animals published by the Japanese government (law no. 105, Oct. 1, 1973). For intracerebroventricular infusion, a guide cannula made of 23-gauge stainless steel tubing was fixed into the cerebral third ventricle 2 wk before the experiments. Three days before the experiments, a chronically indwelling silicone catheter was implanted into the atrium through the right jugular vein and brought out subcutaneously at the back of the neck. To measure mean arterial blood pressure (MAP) and heart rate (HR), a polyethylene catheter (OD 0.8 mm, ID 0.4 mm) was placed into the aorta via the femoral artery and a silicone catheter was implanted into the right atrium via the right jugular vein (both in 28 animals). These catheters were brought out subcutaneously at the back of the neck. When required, the arterial catheter could be connected to a strain gauge pressure transducer (Nihon Kohden PT300T) for recording of raw data to standard pulses with the aid of a window discriminator that separated discharges from background noise. A ratemeter with a reset time of 5 s was used to enable us to follow the time course of the nerve activity. The nerve activity was analyzed by comparing the mean frequency per 5 s over 50-s periods at various times before and after administration of aFGF and by comparing the activity induced by drugs with that recorded after vehicle administration.

Administration of drugs. aFGF and bFGF (R&D Systems) were dissolved in 10 µl aCSF containing 0.1% bovine albumin. Lyophilized anti-CRF antibody (Peptide Institute; containing 50 µl rabbit antiserum against human CRF) was dissolved in 50 µl aCSF. When required, 10 µl of this anti-CRF antibody solution (containing 10 µl antiserum) was administered intracerebroventricularly to an animal 20 min before the start of the experiment.

Measurements. Plasma catecholamines were measured by the coulometric electrochemical determination method, with the minor modification previously reported (19). The minimum detectable concentration of the catecholamines under study was within the range of 3–5 pg/ml. Integrated catecholamine responses (area under the curve) were also calculated after administration of aFGF or vehicle. The integrated responses recorded here represent the increment above basal level over a 180-min period after each application.

Statistical analysis. Data were analyzed by a one- or two-way ANOVA, with a correction for repeated measures, by means of a computer software program designed for statistical analysis (Fisher, Tokyo University, Tokyo, Japan), as reported elsewhere (19). When a significant overall effect was revealed by ANOVA, the significance of differences from the prestimulus value 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 intracerebroventricular administration of aFGF on plasma Epi and NE. An intracerebroventricular administration of 1 or 10 ng/rat aFGF induced

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dose-dependent increases in the plasma levels of both Epi and NE (Fig. 1, A and B). A significant increase in Epi was first detected at 60 min, and the response seemed to be peaking at 150 min. At 180 min after the aFGF administration (when sampling ended), it was showing no sign of returning to the basal level. Similar changes were observed in plasma NE levels. Injection of heat-treated aGF (10 ng) induced no such increases in plasma catecholamines. The integrated responses (over the 180 min after aFGF administration) also showed dose-dependent increases in Epi and NE (Fig. 1, A and B, insets). In absolute terms, the integrated Epi response was more than two times the NE response at each dose of aFGF.

Effects of intravenous administration of aFGF on plasma Epi and NE. Dose-dependent increases in plasma Epi and NE were also observed in response to 1, 10, and 100 ng aFGF given intravenously (Fig. 2, A and B). The plasma Epi level showed a gradual and long-lasting increase and reached a plateau at or before 180 min when 1 or 10 ng aFGF was administered, but, when 100 ng aFGF was administered, the level was still rising at 180 min. Significant increases in NE were evoked from 60 to 120 min or more after intravenous administration of 10 or 100 ng aFGF. Heat-treated aGF (100 ng) had no effect on the level in plasma catecholamines. The integrated responses also showed dose-dependent increases in plasma Epi and NE when aFGF was injected intravenously (Fig. 2, A and B, insets). In absolute terms, the integrated Epi and NE responses to 100 ng aFGF given via the intravenous route were almost the same size as those induced by one-tenth of the dose (i.e., 10 ng aFGF) administered via the intracerebroventricular route.

Effects of intracerebroventricularly or intravenously administered aFGF on MAP and HR. MAP showed a gradual increase with a peak at ~60 min after an
intracerebroventricular administration of 10 ng aFGF. MAP was significantly higher at 60 (132 ± 5.3 mmHg), 90 (134 ± 6.3 mmHg), and 120 (130 ± 9.2 mmHg) min after aFGF administration than at the same times after vehicle injection (Fig. 3A, bottom). In animals given aFGF intracerebroventricularly, HR ranged between 320 and 354 beats/min throughout the study period, whereas in vehicle control animals it was between 310 and 344 beats/min. There was no significant difference between the two groups (Fig. 3A, top). MAP decreased immediately after blood sampling by 3.18 ± 0.98 and 2.40 ± 0.83 mmHg in vehicle- and aFGF-injected groups, respectively (Fig. 3A, inset). After the replacement of blood cells resuspended in saline, MAP increased by 2.25 ± 0.57 and 2.30 ± 0.81 mmHg in vehicle- and aFGF-injected groups, respectively (Fig. 3A, inset). In terms of the changes in MAP (ΔMAP) associated with blood sampling (withdrawal) or blood replacement (replacement) no significant difference was found between aFGF- or vehicle-injected animals (Fig. 3A, inset). When aFGF or vehicle was administered via the intravenous route, no significant changes in MAP or HR were observed throughout the experimental period (Fig. 3B). Again, MAP decreased immediately after blood sampling, this time by 4.88 ± 1.63 and 3.90 ± 1.40 mmHg, in vehicle- and aFGF-injected groups, respectively (Fig. 3B, inset). After the replacement of resuspended blood cells, MAP increased by 5.25 ± 1.43 and 3.98 ± 1.40 mmHg in vehicle- and aFGF-injected groups, respectively (Fig. 3B, inset). In terms of the changes in MAP associated with blood withdrawal or replacement, no significant difference was found between aFGF- and vehicle-injected animals (Fig. 3B, inset).

Effects of aFGF on sympathetic efferent discharge to the adrenal gland. In response to 10 ng icv aFGF, the sympathetic efferent activity in the adrenal nerve showed a clear increase (in terms of the multiunit discharge rate) starting at ~15 min and reaching significance at or before 30 min (Fig. 4A). The mean discharge rate showed a dose-dependent increase, and the increases induced by 1 or 10 ng/rat aFGF were both significantly greater than that induced by vehicle (Fig. 4A, inset). When administered intravenously, 10 ng/rat aFGF again markedly facilitated the discharge (Fig. 4B). By comparison with the basal level, the mean discharge rate at 90 min after the 10 ng/rat administration was increased by ~86% (intracerebroventricularly) or 67% (intravenously) (see Fig. 4, A and B, insets). Administration of vehicle by the intracerebroventricular or intravenous route caused no detectable change in nerve activity.

Effects of aFGF on sympathetic efferent discharge to the spleen and BAT. In the splenic efferent nerves, a significant facilitation of the discharge rate was evoked by an intracerebroventricular administration of 10 ng aFGF, the response beginning at ~38 min and continuing for >180 min (Fig. 5A, top). When 10 ng aFGF was administered via the intravenous route, the discharge rate at first gradually decreased, reaching a minimum level at 30 min, but thereafter gradually increased and showed a significant potentiation from 100 min up to at least 210 min (Fig. 5A, bottom). In response to 10 ng iv aFGF, the discharge rate in the sympathetic efferent nerves to BAT decreased slightly, but not significantly, until ~30 min and then increased markedly up to ~120 min (Fig. 5B). By comparison with vehicle control, the mean discharge rate was significantly increased at 60 (28% above the basal level ), 90 (33%), and 120 (37%) min after the intravenous administration of aFGF (Fig. 5B, inset). No detectable change in nerve activity to the spleen or BAT was found after an administration of vehicle by the intravenous route.

Effects of bFGF on sympathetic efferent discharge to the adrenal gland. In response to 10 ng icv bFGF, the sympathetic efferent activity in the adrenal nerve showed a clear increase at 20 min after the injection, and it reached a plateau level around 90 min. The mean discharge rate increased from 30 to 120 min after the
administration of 10 ng icv bFGF (much as it did after 10 ng icv aFGF) (Fig. 6, A and B). Thereafter, it gradually decreased to the basal level. The mean discharge rate at 210 min was ~23% lower with bFGF than with aFGF (Fig. 6A). When administered intravenously, bFGF again significantly facilitated the sympathetic efferent activity in the adrenal nerve at 60 min after the injection (Fig. 6, A and C). A comparison with the effect on the mean discharge rate induced by the same dose of aFGF showed that although the response induced by intravenous bFGF reached comparable levels to those induced by aFGF at 180 and 210 min after its administration, the mean discharge rates at 60, 90, 120, and 150 min after bFGF administration were, respectively, 19, 16, 14, and 11% lower than those recorded after induced intravenous aFGF (Fig. 6A).

Administration of vehicle by the intracerebroventricular or intravenous route caused no detectable change in nerve activity (Fig. 6, B and C).

Effects of intracerebroventricular or intravenous administration of bFGF on plasma Epi and NE. An intracerebroventricular or intravenous administration of 10 ng/rat bFGF also increased the plasma levels of Epi (Fig. 7A) and NE (Fig. 7B). After an intracerebroventricular injection, a significant increase in Epi was first detected at 60 min. The Epi response peaked at 120 min after aFGF administration and then showed a downward trend. After intravenous administration of 10 ng bFGF, small but significant increases in Epi levels were observed at 40 and 60 min. The integrated responses also showed significant increases in Epi in animals given both intracerebroventricular and intravenous administrations (Fig. 7A, inset). In absolute terms, the integrated Epi response induced by 10 ng icv bFGF was ~32% of that induced by 10 ng icv aFGF, whereas that induced by 10 ng iv bFGF was 22% of the integrated response induced by 10 ng iv aFGF. The plasma NE concentration also showed small but significant increases, and it reached a plateau at 90 min when 10 ng bFGF was administered intracerebroventricularly. After intravenous administration of 10 ng bFGF, small but significant increases in NE were detected at 90 and 120 min. Small but significant increases were also observed in the integrated NE responses in ani-
mals given both intracerebroventricular and intravenous administration (Fig. 7, inset). In absolute terms, the integrated NE response to 10 ng aFGF given intracerebroventricularly (n = 10) or intravenously (n = 10), for 10 ng bFGF given intracerebroventricularly (n = 6) or intravenously (n = 6), and for vehicle intracerebroventricularly (n = 5) or intravenously (n = 5). \#P < 0.05 vs. vehicle control at same time point.

Effects of HVX on the aFGF-evoked increases in sympathetic outflow and plasma Epi and NE. HVX completely prevented the increases in efferent activity in the sympathetic nervous innervating the spleen and adrenal that were evoked in intact animals by adminis-

Effects of aFGF on afferent activity in the hepatic vagus nerve. Afferent activity in the hepatic branch of the vagus increased dose dependently when doses of 10, 50, and 100 ng aFGF were given over 1-min periods into the portal vein (ipv) (Fig. 8A). Administration of vehicle by the portal vein route did not cause any significant change in nerve activity (not shown). Significant increases in activity could be seen at ~10 min after the start of the injections. After 10 or 50 ng aFGF, the response peaked at ~26 min and the firing rate then gradually decreased toward the basal level. When 100 ng aFGF was administered, the increase was extremely long lasting; it had not yet reached a maximal level at 120 min after the injection.

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Fig. 6. Effects of basic FGF (bFGF) on the efferent firing rate in a sympathetic branch supplying the adrenal gland. A: mean discharge rates (± SE) for 10 ng aFGF given intracerebroventricularly (n = 10) or intravenously (n = 10), for 10 ng bFGF given intracerebroventricularly (n = 6) or intravenously (n = 6), and for vehicle intracerebroventricularly (n = 5) or intravenously (n = 5). \#P < 0.05 vs. vehicle control at same time point. B: efferent discharge rates in response to 10 ng icv/rat bFGF (top) or vehicle (bottom). C: efferent discharge rates in response to 10 ng iv/rat bFGF (top) or vehicle (bottom).

Fig. 7. Effect of intracerebroventricular or intravenous bFGF on plasma Epi and NE concentrations. A: plasma Epi before and after administration of vehicle (n = 8) or 10 ng icv/rat bFGF (n = 7) and before and after administration of vehicle (n = 9) or 10 ng iv/rat bFGF (n = 8). Insert: integrated Epi responses to 10 ng bFGF or aFGF given either intracerebroventricularly (left) or intravenously (right). Responses were integrated over 180 min after administration. B: plasma NE before and after administration of vehicle (n = 8) or 10 ng icv/rat bFGF (n = 7) and before and after administration of vehicle (n = 9) or 10 ng iv/rat bFGF (n = 8). Insert: integrated NE responses to 10 ng/rat bFGF or aFGF given either intracerebroventricularly (left) or intravenously (right). Responses were integrated over the 180 min after administration. Values are means ± SE. \#P < 0.05 vs. vehicle, $P < 0.05 vs. 10 ng aFGF.
given 10 ng aFGF by the intravenous route, pretreatment with anti-CRF antibody significantly lowered the plasma levels of Epi and NE evoked by aFGF (Fig. 9, A and B). This effect could be clearly seen by examining the integrated increases in Epi and NE, which showed reductions of 64 and 70.4%, respectively, after anti-CRF antibody treatment (Fig. 9, A and B, insets). Bilateral SPX completely prevented the increases in plasma Epi concentration seen in intact animals after the intravenous (Fig. 9A) or intracerebroventricular (Fig. 10A) administration of 10 ng aFGF. In fact, the integrated

Effects of anti-CRF antibody or SPX on the aFGF-evoked increases in Epi and NE. The basal levels of plasma Epi and NE were not changed significantly by pretreatment with anti-CRF antibody (via the intracerebroventricular route). However, such pretreatment strongly attenuated the aFGF-induced increases in Epi and NE at 90, 120, 150, and 180 min after the injection (Figs. 10, A and B). The integrated increases in both Epi and NE were reduced to about one-third of the responses seen in animals without such pretreatment (Fig. 10, A and B, insets). Furthermore, in animals

given 10 ng aFGF by the intravenous route, pretreatment with anti-CRF antibody significantly lowered the plasma levels of Epi and NE evoked by aFGF (Fig. 9, A and B). This effect could be clearly seen by examining the integrated increases in Epi and NE, which showed reductions of 64 and 70.4%, respectively, after anti-CRF antibody treatment (Fig. 9, A and B, insets). Bilateral SPX completely prevented the increases in plasma Epi concentration seen in intact animals after the intravenous (Fig. 9A) or intracerebroventricular (Fig. 10A) administration of 10 ng aFGF. In fact, the integrated

Fig. 8. Effect of portal vein administration (ipv) of aFGF on afferent firing rate in the hepatic branch of the left vagus nerve (A) and effect of hepatic vagotomy (HVX) on the increase in efferent firing rate of sympathetic branches supplying the spleen (B; top) or adrenal gland (B; bottom). Responses in B were evoked by aFGF given intravenously (top) or into the portal vein (bottom).
In this study, it was shown that not only exogenous aFGF but also exogenous bFGF induced activations of sympathetic outflow and adrenomedullary secretion, although aFGF was more effective than bFGF. This observation is consistent with our previous finding that aFGF has a suppressant effect on feeding behavior that is about twofold stronger than that of bFGF (8) and with the observation reported by Knefati et al. (14) that aFGF, but not bFGF, has somnogenic and thermogenic actions. In the present study, heat treatment completely abolished the effects otherwise induced by aFGF. Although after blood withdrawal MAP decreased by 3.18 ± 0.98 and 4.88 ± 1.63 mmHg, after blood replacement it increased again by 2.25 ± 0.57 and 5.25 ± 1.43 mmHg (in animals given aFGF via the intracerebroventricular and intravenous routes, respectively). In vehicle-injected animals, the changes in MAP after blood sampling or replacement were comparable to those seen in aFGF-injected animals. In fact, there were no statistical differences among the decreases in MAP or among the increases whether rats were given vehicle, intracerebroventricular aFGF, or intravenous aFGF. A long-lasting activation of the sympathetic efferent nerves innervating the adrenal, spleen, and BAT accompanied the increase in MAP observed in animals given aFGF intracerebroventricularly, but not in vehicle-injected animals. Thus it is unlikely that these increases in sympathetic efferent activity and in plasma catecholamines were secondary to any changes in MAP associated with blood sampling and/or replacement. The activation of sympathetic outflow and the increased adrenomedullary secretion induced by aFGF seem to be specific responses to aFGF itself.

aFGF administered intracerebroventricularly induced dose-dependent increases not only in the plasma levels of Epi and NE but also in the efferent firing rate in the sympathetic adrenal nerves in our anesthetized rats. Doses of 1 and 10 ng icv would give final concentrations in the CSF of ~0.2 and 2.1 pmol/ml (CSF volume: 300 µl). These concentrations are within the physiological range, because the basal aFGF level in the CSF is 0.7 pmol/ml and the level increased to 0.7 nmol/ml at 15 min and 7.5 nmol/ml at 45 min after food intake or after 4 mM icv glucose application (8, 25). The most important results in the present intracerebroventricular experiments were as follows. The increases in plasma Epi and adrenal efferent nerve activity persisted for up to 180 min after an intracerebroventricular application of aFGF. The integrated Epi response induced by 10 ng icv aFGF was some 2.5 times greater than the corresponding NE response. Bilateral SPX completely abolished the increase in Epi but not the increase in NE. On
the other hand, pretreatment with anti-CRF antibody via the intracerebroventricular route significantly attenuated not only the increases in Epi evoked by 10 ng aFGF via the intracerebroventricular and intravenous routes but also the increases in NE. In fact, the integrated increases in Epi and NE were both reduced to ~30% of those seen in the intact animal.

On intravenous administration of aFGF, marked and long-lasting increases in Epi and NE were again observed, together with increases in adrenal sympathetic efferent activity. The injected aFGF doses of 1, 10, and 100 ng would give final plasma concentrations of 6.3, 63, and 630 fmol/ml if the rat’s plasma volume is assumed to be 11 ml on average. These are also within the physiological range. When given intravenously, aFGF caused a facilitation of sympathetic efferent activity not only to the adrenal gland but also to the spleen and BAT. The integrated Epi response was ~3.4 times the corresponding NE response. In this study, aFGF was significantly less potent when injected intravenously than when injected centrally in both evoking increases in the integrated Epi and NE responses (67% and 48.3%, respectively, of the responses to intracerebroventricular aFGF) and facilitating the efferent activity in the sympathetic adrenal nerves (82.3% of the intracerebroventricular response at 90 min after the injection). The effects of SPX on the integrated increases in Epi and NE were different: the former were completely abolished, whereas the latter were reduced by one-half or less (the response to intravenous aFGF being affected much less than that to intracerebroventricular aFGF). Pretreatment with anti-CRF antibody, which attenuated the increases in Epi and NE by 62 and 68%, respectively, when aFGF was administered centrally, attenuated them by 71 and 68% when aFGF was given peripherally. HVX abolished almost completely not only the integrated increases in Epi and NE but also the intravenous aFGF-induced potentiation of the sympathetic efferent outflow to the adrenal, spleen, and BAT. Recently, we reported that microelectrophoretic application of aFGF to parvocellular neurons of the PVN in vitro increased neuronal activity in more than one-third of the neurons tested (30). It may be that aFGF administered centrally potentiates the sympathoadrenomedullary axis via an activation of parvocellular CRF neurons.

Interestingly, marked increases in the plasma corticosterone level have been found after intravenous or intracerebroventricular administration of aFGF (18). However, pretreatment with an intracerebroventricular injection of anti-CRF antibody reduced the integrated increases in corticosterone by ~60% when aFGF was administered centrally but by only 25% (not significantly) when it was administered peripherally (18). In the same study, peripheral aFGF application activated the pituitary and provoked ACTH release (18) and HVX did not affect the corticosterone increase when aFGF was administered peripherally. These observations and the present ones indicate that 1) the activation of the sympathoadrenomedullary axis by peripherally administered aFGF occurs in a quite different way from the activation of the hypothalamic-pituitary-adrenal axis, and 2) when aFGF is administered systemically, hepatic afferent impulses lead to increased CRF release in the brain. Ultimately, the released CRF would lead to activation of the sympathetic outflow and increases in Epi and NE via the intermediolateral column, the origin of the efferent sympathetic motoneurons. Possibly, hepatic afferent signals may be conveyed into the nucleus of the solitary tract (NTS) and the neuronal input from the NTS to the PVN may then trigger CRF release. In any event, centrally released CRF after intravenous or intracerebroventricular administration of aFGF seems likely to play a key role in the activation of the hypothalamic-sympatho-adrenomedullary axis.

It is well known 1) that food intake increases both sympathetic outflow (2, 13, 32) and urinary catecholamine excretion (6, 12) and 2) that feeding or glucose ingestion increases thermogenesis through an activation of BAT (22, 26, 32). In the present study, aFGF induced parallel increases in the sympathetic efferent activity to the adrenal and BAT. In general, the firing rate of the sympathetic efferent nerves is reciprocally related to the blood glucose level (4, 21). By comparison with the response in BAT, an inverse response was found in the adrenal efferent nerve activity within 30 min after an intravenous injection of 300 mg/kg glucose (21, 22). Thus whether the increases in urinary catecholamine excretion and thermogenesis after feeding or glucose ingestion are mediated by aFGF-induced adrenomedullary activation remains unknown. The parallel activation of the sympathetic outflow to the adrenal and BAT may show that aFGF has a multifunctional role in the control of neuronal and/or endocrinological activity. Indeed, Kniefati et al. (14) suggested that aFGF forms part of a complex cytokine network in the brain, because intracerebroventricular aFGF but not bFGF induces non-REM sleep and fever in rabbits (14). Intravenous administration of aFGF (100 ng/rat) also induces a long-lasting hyperthermia of ~1°C in awake and freely moving rats (our unpublished observation). These results suggest that the long-lasting fever induced by exogenous aFGF given via the intravenous or intracerebroventricular route is caused, at least in part, by activation of sympathetic nerves innervating the adrenal medulla and BAT via the release of CRF in the brain.

Perspectives

A further area of interest is the link between aFGF and the immune system. In the present study, exogenous aFGF activated the sympathetic outflow to the spleen and adrenal medulla, and it also activates the hypothalamic-pituitary-adrenal axis (18). Splenic natural killer cell cytotoxicity is reduced by activation of the splenic sympathetic outflow (with associated increases in plasma NE concentration), which is in turn evoked via CRF release in the rat brain (10, 31). Furthermore, it has become clear that catecholamines, as well as glucocorticoids, should be viewed as physiological inhibitors of inflammatory responses and as immunosuppressive mediators (33). These data suggest that aFGF
would be expected to affect the immune system. Indeed, exogenous aFGF application protects against degeneration of hippocampal CA1 neurons after experimental brain ischemia (28) and attenuates the impairment of immune functions otherwise seen in aged senescence-accelerated P8 mice (24).

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REFERENCES


