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A non peptidic corticotropin releasing factor receptor antagonist attenuates fever and exhibits anxiolytic-like activity

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Abstract

The multiple actions of corticotropin-releasing factor (CRF) on neuroendocrine and behavioural functions can now be examined using new, high affinity, non peptidic antagonists which exhibit central activity upon systemic application. We have shown that compound CP 154,526 (butyl-ethyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]amine) displaces [^{125}I][Tyr^0]CRF from rat hippocampal CRF receptors (IC_{50} = 0.5 nM) and from pituitary CRF receptors (IC_{50} = 0.04 nM). The same compound inhibits in a concentration-dependent manner the ovine CRF (0.1 μM)-stimulated adenylate cyclase activity in membranes of a mouse pituitary adenoma cell line, AtT20, with an IC_{50} value of 50 nM. Systemic application of the CRF receptor antagonist (0.16 mg/kg i.p.) blocked recombinant human interleukin-1β (5 μg/kg i.p.) induced fever in rats. The CRF receptor antagonist CP 154,526 (1 mg/kg i.p.) also exhibited signs of anxiolytic-like activity in the elevated plus-maze test in rats.

Keywords: CRF (corticotropin-releasing factor); Anxiety; Interleukin-1β; Fever; CRF receptor antagonist

1. Introduction

Corticotropin-releasing factor (CRF) is a 41 amino acid long neuropeptide with wide spread distribution in the central nervous system (Vale et al., 1981; Merchenthaler et al., 1982; Swanson et al., 1983). It is one of the key regulators of the stress response via its stimulatory effects on the release of adrenocorticotropic hormone (ACTH) and of propiomelanocortin products from the anterior pituitary (Chappell et al., 1986; Fisher, 1991). CRF neurons also innervate the limbic system and brain stem, and appear to participate in the control of sympathetic outflow and metabolic rates (Brown et al., 1982). CRF has been implicated in anxiety using different models such as cocaine withdrawal induced anxiety (Sarnyai et al., 1995), social defeat-induced anxiety (Skutella et al., 1994) and in elevated plus-maze behaviour. Furthermore CRF over producing transgenic mice show increased anxiety (Stenzel-Poore et al., 1994). CRF receptors are wide spread as shown by receptor autoradiography using [^{125}I][Tyr^0]CRF as tracer (De Souza et al., 1984; Webster and De Souza, 1988). CRF receptor subtypes have recently been cloned and the subtype dominating in the pituitary, olfactory bulb and cerebral cortex has been designated as CRF-R_1, while the subtype occurring in the septum, hypothalamus and brain stem has been designated as CRF-R_2, respectively (Chen et al., 1993; Lovenberg et al., 1995). Both receptor subtypes belong to the super family of G-protein-coupled receptors with seven putative transmembrane domains, and both receptor subtypes, when occupied by agonists, stimulate adenylate cyclase activity in the appropriate tissues via G_s protein coupling.

Recently, some high affinity, non peptidic CRF receptor ligands have been reported (Hagan, 1995).

These ligands represent an important step towards understanding the physiology of the CRF funtions as they afford selective and strong blockade of central CRF receptors upon systemic administration. The only CRF receptor antagonists available earlier were long peptide ligands; the

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α-helical 9–41 CRF receptor antagonist (Rivier et al., 1984) and D-Phe CRF12–41 (Menzaghi et al., 1994), which had to be injected intracerebroventricularly when studying CNS effects of CRF and CRF receptor antagonists. This problem made many studies, particularly those on stress responses, cumbersome. The effects of the high affinity non peptidic CRF receptor ligand CP 154,526 (butyl-ethyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]amine) are reported here on in vitro experiments using binding and second messenger studies, and on in vivo assays on anxiety and fever, respectively.

2. Materials and methods

2.1. Materials and experimental animals

The radiochemicals [^{125}I][Tyr^0]ovine CRF (2200 Ci/mmol) and [^{3}H]cAMP (28.20 Ci/mmol) were purchased from Du Pont, NEN. Ovine CRF was bought from Sigma and the CRF receptor ligand CP 154,526 (butyl-ethyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo-[2,3-*d*]pyrimidin-4-yl]amine) (molecular weight = 401) was synthesised by Dr U. Widmer at Hoffmann La Roche, Basel, Switzerland. The recombinant human interleukin-1β (rhIL-1β, 10^7 U/mg) was a kind gift from Sclavo (Siena, Italy). Reagents for the adenylate cyclase measurements were purchased from Sigma and Boeringer Mannheim apart from the binding protein which was purified from bovine adrenal medullas (Brown et al., 1972). The AtT-20 cells were from American Type Culture Collection (ATCC). Cell culture medium and supplements were bought from Life Technologies. The radio telemetry equipment was purchased from Mini Mitter, Sunriver, OR. The male Sprague Dawley rats for the fever measurements and binding studies were from ALAB and Eklunds, Sweden, respectively. Male Sprague Dawley rats for the plus-maze experiment were obtained from Biological Research laboratories, Switzerland.

2.2. Binding studies

Male Sprague Dawley rats (200 g) were decapitated and the hippocampi and pituitaries were rapidly dissected. Membranes were prepared by homogenizing the tissues (10% w/v) in 0.32 M sucrose solution buffered with 5 mM Hepes (pH 7.4) followed by centrifugation at $100 \times g$ for 10 min. The supernatant was subjected to further centrifugation at $10\,000 \times g$ for 45 min. The resulting pellet (P2) was resuspended in bacitracin containing (1 mg/ml) Krebs-Ringer solution consisting of 5 mM Hepes, 137 mM NaCl, 2.68 mM KCl, 2.05 mM $MgCl_2$, 1.8 mM $CaCl_2$, 1 g/l glucose and 0.05% (w/v) bovine serum albumin, pH 7.4.

Displacement studies on the freshly prepared hippocampal or pituitary membranes were carried out with [^{125}I][Tyr^0]ovine CRF (0.1–0.2 nM) as radiolabelled ligand and up to 1 μM of ovine CRF or compound CP 154,526 as displacers. The binding of [^{125}I][Tyr^0]ovine CRF was determined using a centrifugation method (De Souza et al., 1985). The experiments were carried out in bacitracin containing (1 mg/ml) Hepes-buffered Krebs-Ringer solution (pH 7.4) in a final volume of 300 μl. Assays were started by the addition of membranes. The incubations were carried out at 22°C for 2 h and they were stopped by centrifugation at $12\,000 \times g$ for 5 min. The pellets were gently washed three times with 1 ml of ice-cold phosphate buffered saline (PBS), pH 7.2, containing 0.01% (v/v) Triton-X followed by centrifugation at $12\,000 \times g$ for 5 min. The bottoms of the Eppendorf tubes were cut off and the radioactivity measurements were carried out in a Packard gamma counter. The IC_{50} values for the cold ligands were calculated using the non linear least squares method of the program KaleidaGraph (Macintosh). The Hill coefficient of the displacement curve was determined by the same program.

2.3. Adenylate cyclase measurements

Cells of the mouse anterior pituitary cell line AtT-20 were cultivated in F-10 Nutrient Mixture (Ham) medium containing 15% (v/v) horse serum, 2.5% (v/v) fetal calf serum, 100 units/ml penicillin and 100 mg/ml streptomycin. The cells (passage 59–60) were harvested in growth phase and cell membranes were prepared by lysis of the cells in 5 mM Tris-HCl pH 7.4 followed by centrifugation ($1600 \times g$) for 15 min at +4°C. Freshly prepared cell membranes were incubated with 100 nM ovine CRF and various concentrations of the compound CP 154,526 (10 nM to 3 μM, Fig. 2) at 30°C for 10 min in a 150 μl reaction volume consisting of 30 mM Tris-HCl, 8.25 mM $MgCl_2$, 1.5 mM theophyllin, 0.75 mM EGTA, 0.1 mM NaCl, 7.5 mM KCl, 10 mM phosphoenolpyruvate, 30 μl/ml pyruvatkinase, 100 μg/ml bacitracin, 20 μg/ml aprotin, 1 mM ATP and 10 μM GTP, pH 7.4. The incubations, all performed in triplicates, were stopped by adding EDTA to a final concentration of 25 mM followed by boiling the samples for 3 min. The amount of cAMP produced was measured using the regulatory subunit of the cAMP dependent protein kinase of the bovine adrenals in a competitive binding assay according to Brown et al. (1972). The experiment was performed twice.

2.4. Fever measurements

Sprague Dawley male rats (220–250 g, 50–60 days old) were housed in single plexiglass cages, under a 12 h light/12 h dark cycle (light from 8:00 a.m. to 8:00 p.m.), and maintained with lab chow and water ad libitum. The temperature in the room was constantly kept at 27 ± 1°C and the humidity was about 50–60%. All animals were housed at 27 ± 1°C for at least 3 days before starting the

experiment. A radiotransmitter was implanted inside the peritoneum, under anaesthesia with pentobarbital sodium (60 mg/kg, i.p.), 5 days before the day of recombinant human interleukin-1β (5 μg/kg, i.p.) and CP 154,526 (0.16–0.8 mg/kg, i.p.) injections (CP 154,526 was dissolved in saline containing 1.5% ethanol). Four days after the surgery, the body weight of all the animals with a radio transmitter inserted in the peritoneum was indistinguishable from that of normal rats of the same age.

The core body temperature of the animals were measured using battery-operated biotelemetry devices. Each radio transmitter was calibrated and wax coated before implantation (final weight: 3 g). Output (frequency in Hz for the body temperature) was monitored by a receiver placed under each animal's cage and fed into a processor connected to an IBM personal computer. Body temperature values were recorded at 10 min intervals beginning at least 24 h before the start of injection and continued for at least 48 h after it. All the injections were performed at 10:00–11:00 in the morning.

2.5. Test of anxiolytic activity

The CRF receptor antagonist CP 154,526 was tested in a standard rat plus-maze placed in a closed isotropic and soundproof environment with controlled atmospheric conditions. Thirty minutes following drug administration, the following behavioural parameters were scored via a closed-circuit TV camera for 5 min: time spent in open arms, number of transitions from closed to open arms, and from closed to closed arms. In addition, 5 min after completion of the plus-maze experiment, rats were submitted to an horizontal wire test for evaluation of neuromuscular function. Placebo versus three doses were counterbalanced and administered intraperitoneally to groups of 16 male Sprague Dawley rats per dose. (CP 154,526 was dissolved in saline containing 0.3% v/v Tween 80.)

3. Results

The non peptidic CRF receptor ligand CP 154,526 displaced [^{125}I][Tyr^0]ovine CRF from rat hippocampal membranes with IC_{50} value of 0.5 nM and from pituitary membranes with IC_{50} value 0.04 nM (Fig. 1a,b). It should be noted that [^{125}I][Tyr^0]ovine CRF binds itself with different affinity to hippocampal ($IC_{50} = 3.3$ nM) and to pituitary ($IC_{50} = 7.5$ nM) membranes, respectively. The difference in the affinity of CP 154,526 was however greater between these two tissues (and receptor subtypes). It should also be noted that the Hill coefficient of displacement of [^{125}I][Tyr^0]ovine CRF by CP 154,526 from pituitary receptors was lower than unity ($n = 0.74$), suggesting possible existence of a high and low affinity binding site, respectively.

Compound CP 154,526 inhibited the ovine CRF (100 nM) stimulated adenylate cyclase activity in membranes from AtT20 murine pituitary adenoma cells with an IC_{50} value of about 50 nM (Fig. 2).

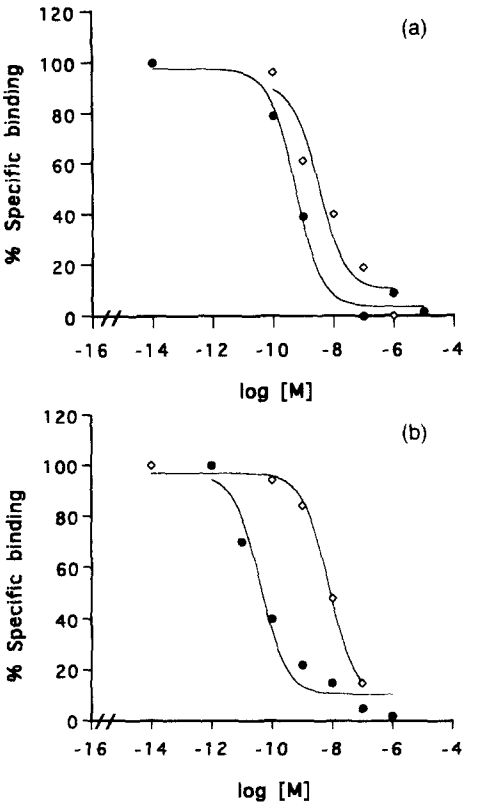

Fig. 1. (a) Displacement of [^{125}I][Tyr^0]ovine CRF by ovine CRF (◇) and CP154,526 (●) in the membranes from the hippocampus. Each point is expressed as the percentage of specific binding. The concentration of [^{125}I][Tyr^0]ovine CRF was 0.1–0.2 nM. The specific binding was 30–35% of the total binding in each experiment. (b) Displacement of [^{125}I][Tyr^0]ovine CRF by ovine CRF (◇) and CP154,526 (●) in the membranes of the pituitary. Each point represents the mean data obtained from two experiments, each point performed in duplicate and expressed as the percentage of specific binding. The concentration of [^{125}I][Tyr^0]ovine CRF was 0.1–0.2 nM. The specific binding was more than 85% of the total binding in each experiment.

The non peptidic CRF antagonist CP 154,526 also significantly attenuated interleukin-1β induced fever in rats when injected intraperitoneally (0.16–0.8 mg/kg) 15 min prior to the administration of recombinant human interleukin-1β (5 μg/kg i.p.) (Fig. 3). The blockade of the fever response was at the lower dose almost complete and did not increase at the higher dose (data not shown). Saline (containing 1.5% ethanol) injection followed by saline injection as control to CP 154,526 injection followed by saline injection gave no change in body temperature except for the handling peak, which occurred with all injections, saline as well as CP 154,526, and thus was not caused or modified by this compound (data not shown).

The intraperitoneal injection of the non peptidic CRF receptor antagonist affected behaviour in elevated plus-maze test in rats (Fig. 4). Three doses of CP 154,526 (1, 3 and 10 mg/kg i.p.) administered versus vehicle, induced a

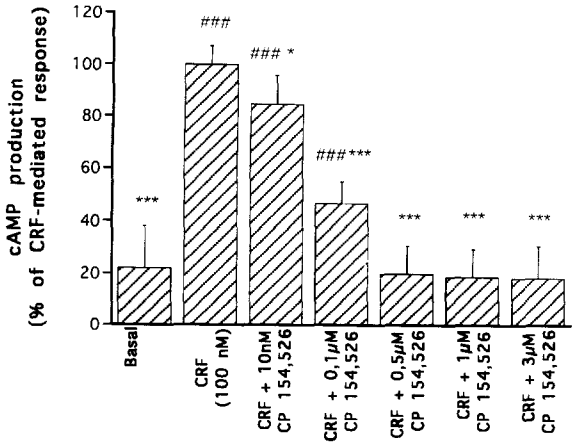

Fig. 2. Blockade of the ovine CRF mediated cAMP production by CP 154,526 in membranes from the mouse anterior pituitary cell line AtT-20. The concentration of CRF was 100 nM. Data are presented as comparisons of different treatments to CRF treatment alone, where CRF treatment alone is set to 100%. $n = 6$ and diagram shows mean + S.E., $^{###}$ $P < 0.001$ versus basal, *** $P < 0.001$ and * $P < 0.05$ versus CRF (100 nM). The data were analysed by ANOVA followed by Fisher's protected least significant difference test.

statistically significant increase in the time spent in the open arms [$F(3,63) = 4.53$, $P < 0.01$] and in the number of transitions from closed to open arms [$F(3,63) = 5.90$, $P < 0.01$]. Indeed, the dose of 1 mg/kg induced a 170% increase in the time spent in the open arms and a 190% increase in the transitions from closed to open arms (unpaired Student's t-test, $P < 0.01$ and $P < 0.001$), respec-

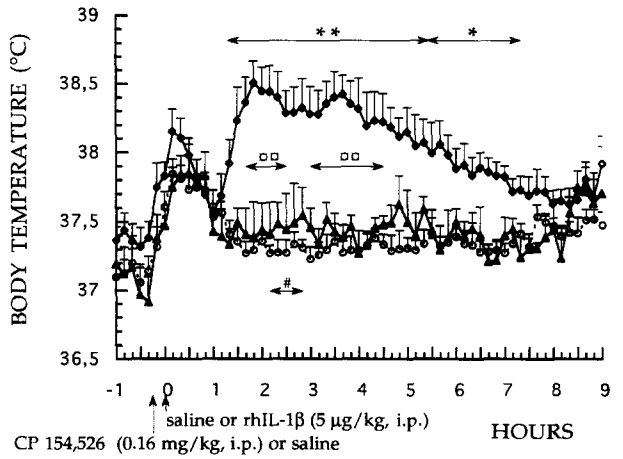

Fig. 3. Blockade of recombinant human interleukin-1β (5 μg/kg, i.p.) induced fever by CP 154,526 (0.16 mg/kg, i.p.) injected 15 min prior to the interleukin-1β administration. Data were recorded every 10 min (mean + S.E.) and analysed by ANOVA followed by Fisher's protected least significant difference test. Rats pretreated with CP 154,526 prior to saline (o, $n = 8$); rats pretreated with CP 154,526 prior to interleukin-1β (p, $n = 4$); rats pretreated with saline prior to interleukin-1β (▲, $n = 8$). ** $P < 0.01$ and * $P < 0.05$ saline + interleukin-1β-injected rats versus CP 154,526 + saline-injected rats; $^{□□}$ $P < 0.01$ saline + interleukin-1β-injected rats versus CP 154,526 + interleukin-1β-injected rats; $^{\#}$ $P < 0.05$ CP 154,526 + interleukin-1β-injected rats versus CP 154,526 + saline-injected rats.

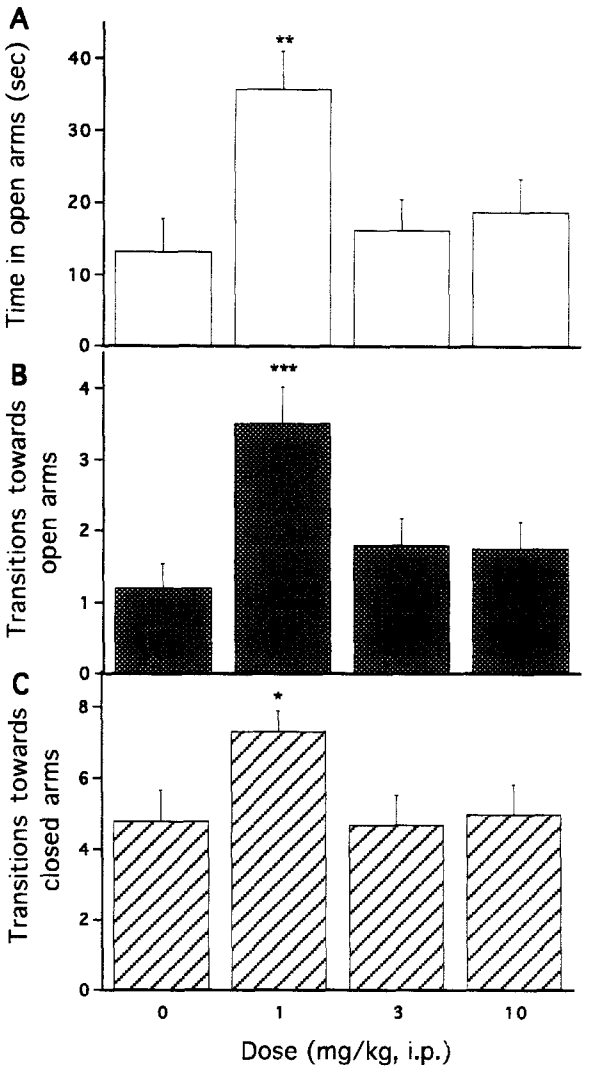

Fig. 4. Anxiolytic-like effects of CP 154,526 administered i.p. 30 min before testing on the elevated plus-maze. (A) Time spent in the open arms; (B) number of transitions towards open arms; and (C) number of transitions towards closed arms, data presented show mean + S.E.M.. Significantly different from vehicle-treated rats: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Data were analysed by single factor ANOVA followed by unpaired Student's t-test for individual dose comparisons versus placebo.

tively. A 52% increase in transitions from closed to closed arms (unpaired Student's t-test, $P < 0.05$) reflected the behavioural disinhibition observed with this dose in the animals. These effects were not dose-dependent since higher doses (3.2 and 10 mg/kg i.p.) did not induce significant effects (42%, 50% and 4% increase in the time spent in the open arms, number of transitions from closed to open arms, and number of transitions from closed to closed arms, respectively, following the administration of 10 mg/kg i.p., unpaired Student's t-test, $P > 0.05$). Lower doses were not tested. These data suggest that signs of anxiolytic-like activity, reminiscent of the anxiolytic-like effects of benzodiazepines in this test, are observed at moderate doses of compound CP 154,526. Those signs

were not observed at higher doses, possibly resulting from interfering non specific interactions at high doses with this drug. No motor impairment was observed in the 1–10 mg/kg i.p. dose range.

4. Discussion

Corticotropin-releasing factor plays an important role in neuro-immune interactions as a key mediator of the hypothalamus-pituitary-adrenal axis in response to stress, infection and inflammation (Rivier and Plotsky, 1986; Swanson et al., 1986). CRF also activates the sympathetic outflow and has a number of metabolic effects (Brown et al., 1982). In addition the anxiogenic, mood modifying properties of CRF are well documented (Koob et al., 1993).

The cloning of the cDNA for CRF receptor subtypes (Chen et al., 1993; Lovenberg et al., 1995) and the identification of high affinity peptidic ligands (Menzaghi et al., 1994) and non peptidic ligands (Hagan, 1995) to the CRF receptors have brought in the past two years the CRF system into focus of neuro immunology and psycho pharmacology.

The present study shows that the high affinity compound CP 154,526 is a CRF receptor antagonist active both in vitro and in vivo. The compound exhibits about twelve times higher affinity for pituitary CRF receptors than for CRF receptors in the hippocampus (Fig. 1) (Chen et al., 1993).

The known involvement of endogenous CRF in anxiety is here reflected by the anxiolytic like effect of this CRF receptor antagonist in the rat elevated plus-maze test. This is in good agreement with other data which have shown that this compound blocked CRF-induced potentiation of startle and fear responses (Hagan, 1995). We have at the present time no adequate explanation to the lack of anxiolytic effects at higher doses than of 1 mg/kg.

This non peptidic CRF receptor ligand is also a potent inhibitor of the fever response to the endogenous pyrogen interleukin-1β (IL-1β). In fact it appears to be a more potent antagonist of pyrogenic than of anxiogenic effects, in these two models.

The fever responses to interleukin-1β but not to lipopolysaccaride (LPS) or tumor necrosis factor α (TNFα) were earlier shown to be attenuated by the i.c.v. injection of α-helical CRF receptor antagonist peptide or by the i.c.v. injection of CRF-antibodies (Rothwell, 1989). This suggests that CP 154,526 crosses the blood brain barrier since this compound, when injected intraperitoneally, mimicks the effects of i.c.v. injection of the peptide-type α-helical CRF receptor antagonist, although direct evidence for appearence of CP 154,526 in the brain is not yet available. Part of the fever blocking effects of CP 154,526 could also be exerted at the peripheral level as it was also shown that in rabbits peripheral treatment with antibodies to CRF, or with α-helical CRF receptor antagonist, can block the fever induced by poly I poly C (Milton et al., 1993).

It is likely that the mode of action of the CP 154,526-mediated anxiolytic and anti pyrogenic effects also involves the down regulation of the sympathetic outflow by inhibiting the cAMP release, required for CRF-mediated activation of sympathetic neurons (Brown et al., 1982). Indeed CP 154,526 potently antagonises the CRF-activation of adenylate cyclase in membranes of a pituitary adenoma cell line, AtT-20, with the IC_{50} value of 50 nM (Fig. 2).

In summary the non peptidic high affinity CRF receptor antagonist, CP 154,526, with a 12-fold preference for rat pituitary CRF receptors over rat hippocampal CRF receptors, reduces both anxiety and fever responses in rats.

References

Brown, B.L., R.P. Ekins and J.D.M. Albano, 1972, Saturation assay for cyclic AMP using endogenous binding protein, Adv. Nucl. Res. 2, 25.

Brown, M.R., L.A. Fisher, J. Spiess, C. Rivier, J. Rivier and W. Vale, 1982, Corticotropin-releasing factor: Actions on the sympathetic nervous system and metabolism, Endocrinology 111, 928.

Chappell, P.B., M.A. Smith, C.D. Kilts, G. Bissette, J. Ritchie, C. Anderson and C.B. Nemeroff,1986, Alteration in corticotropin-releasing factor like immunoreactivity in discrete rat brain regions after acute and chronic stress, J. Neurosci. 6, 2908.

Chen, R., K.A. Lewis, M.H. Perrin and W.W. Vale, 1993, Expression cloning of a human corticotropin-releasing factor receptor, Proc. Natl. Acad. Sci. USA 90, 8967.

De Souza, E.B., M.H. Perrin, T.R. Insel, J. Rivier, W. Vale and M.J. Kuhar, 1984, Corticotropin-releasing factor receptors in rat forebrain: autoradiographic identification, Science 224, 1449.

De Souza, E.B., T.R. Insel, M.H. Perrin, I. Rivier, W.W. Vale and M.J. Kuhar, 1985, Corticotropin-releasing factor receptors are widely distributed within the rat central nervous system: an autoradiographic study, J. Neurosci. 5, 3189.

Fisher, L.A., Corticotropin-releasing factor and autonomic-cardiovascular responses to stress, 1991, in: Stress, Neuropeptides and Systemic Disease, eds. J.A. McCubbin, P.G. Kaufmann and C.B. Nemeroff (Academic Press, San Diego) p. 95.

Hagan, J.J., 1995, The 5th Summer Neuropeptide Conference, Exp. Opin. Invest. Drugs 4, 881.

Koob, G.F., S.C. Heinrichs, E.M. Pich, F. Menzaghi, H. Baldwin, K. Miczek and K.T. Britton, The role of corticotropin-releasing factor in behavioural responses to stress, 1993, in: Corticotropin-Releasing Factor, eds. D.J. Chadwick, J. Marsh and K. Ackrill, Ciba Found Symp. 172, 277.

Lovenberg, T.W., C.W. Liaw, D.E. Grigoriades, W. Clevenger, D.T. Chalmers, E.B. De Souza and T. Oltersdorf, 1995, Cloning and characterization of a functionally distinct corticotropin releasing factor receptor subtype from rat brain, Proc. Natl. Acad. Sci. USA 92, 836.

Menzaghi, F., R.L. Howard, S.C. Heinrichs, W. Vale, J. Rivier and G.F. Koob,1994, Characterization of a novel and potent corticotropin releasing factor antagonist in rats, J. Pharmacol. Exp. Ther. 269, 564.

Merchenthaler, I., S. Vigh, P. Petrusz and A.V. Schally, 1982, Immunocytochemical localization of corticotropin-releasing factor (CRF) in the rat brain, Am. J. Anat. 165, 385.

Milton, N.G.N., E.W. Hillhouse and A.S. Milton, 1993, A possible role for endogenous peripheral corticotropin-releasing factor-41 in the febrile response, J. Physiol. 465, 415.

Rivier, C.L. and P.M. Plotsky, 1986, Mediation by corticotropin releasing factor (CRF) of adenohypophysial hormone secretion, Ann. Rev. Physiol. 48, 475.

Rivier, J., C. Rivier and W. Vale, 1984, Synthetic competitive antagonist of corticotropin releasing factor. Effect on ACTH secretion in the rat, Science 224, 889.

Rothwell, N.J., 1989, CRF is involved in the pyrogenic and thermogenic effects of IL-1β in the rat, Am. J. Physiol. 256, E111.

Sarnyai, Z., E. Biro, J. Gardi, M. Vecsernyes, J. Julesz and G. Telegdy, 1995, Brain corticotropin-releasing factor mediates 'anxiety-like' behaviour induced by cocaine withdrawal in rats, Brain Res. 675, 89.

Skutella, T., A. Montkowski, T. Stohr, J.C. Probst, R. Landgraf, F. Holsboer and G.F. Jirikowski, 1994, Coticotropin-releasing hormone (CRH) antisense oligodeoxynucleotide treatment attenuates social defeat-induced anxiety in rats, Cell. Mol. Neurobiol.14, 579.

Stenzel-Poore, M.P., S.C. Heinrichs, S. Rivest, G.F. Koob and W.W. Vale, 1994, Overproduction of corticotropin-releasing factor in transgenic mice: a genetic model of anxiogenic behavior, J. Neurosci. 14, 2579.

Swanson, L.W., P.E. Sawchenko, J. Rivier and W.W. Vale, 1983, Organization of ovine corticotropin releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study, Neuroendocrinology 36, 165.

Swanson, L.W., P.E. Sawchenko and R.W. Lind, 1986, Regulation of multiple peptides in CRF parvocellular neurosecretory neurons: Implications for the stress response, Prog. Brain Res. 68, 169.

Vale, W., J. Spiess, C. Rivier and J. Rivier, 1981, Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin, Science 213, 1394.

Webster, E.L. and E.B. De Souza, 1988, Corticotropin releasing factor receptors in mouse spleen: identification, autoradiographic localization and regulation by divalent cations and guanine nucleotides, Endocrinology 122, 609.