Effects of \( \alpha_2 \)- and \( \beta \)-Adrenoceptor Agonists on Growth Hormone Secretion Following Lesion of the Noradrenergic System of the Rat

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The aim of the present investigation was to lesion the noradrenergic system and to measure the effect on growth hormone (GH) secretion following peripheral administration of \( \alpha_2 \)- and \( \beta \)-adrenoceptor agonists. Direct injection of these agonists into the paraventricular nucleus of the hypothalamus (PVN) and its effect on GH secretion were also investigated. Systemic administration of N-2-chloroethyl-N-ethyl-2-bromobenzylamine (DSP\(_4\), 60 mg/kg, injected i.p. 10 days prior to experimentation) significantly decreased the noradrenaline (NA) content of the hippocampus, frontal cortex and hypothalamus but had no effect on the dopamine (DA) or serotonin (5-HT) content of these areas. Bilateral injection of 6-hydroxydopamine (6-OHDA, 10 \( \mu \)g/\( \mu \)l, 14 days prior to experimentation) into the medial forebrain bundle (MFB) caused a greater reduction of NA and also decreased the DA and 5-HT content of the hypothalamus. Analysis of the PVN of the hypothalami of rats following 6-OHDA lesion of the MFB showed significantly decreased NA and 5-HT content. Neither DSP\(_4\) treatment nor 6-OHDA lesion of the MFB affected the clonidine (250 \( \mu \)g/kg, i.p.) induced stimulation of GH secretion. Injection of isoproterenol (1 mg/kg, i.p.) had varying effects on GH secretion. It stimulated GH release in control rats but not in DSP\(_4\) or MFB lesioned rats. Direct injection of clonidine (0.1 \( \mu \)g/\( \mu \)l) into the PVN significantly stimulated GH secretion, whereas injection of isoproterenol (2.5 \( \mu \)g/\( \mu \)l) into the PVN did not affect GH levels when compared to controls. The results of the present study do not support the hypothesis that hypoactivity of the central noradrenergic system may be the cause of the blunted GH response to clonidine observed in depressed patients.

KEY WORDS: GH; DSP\(_4\); 6-OHDA; MFB; lesion; clonidine; isoproterenol; PVN.

INTRODUCTION

Growth hormone (GH) release is regulated by non-competitive antagonism between growth hormone releasing hormone (GHRH) and somatostatin (1,2). The regulatory effects of GHRH seem to be specific for GH release (1). In addition to the neurohormones, several brain neurotransmitters have been shown to influence GH secretion, having both stimulatory and inhibitory effects which are exerted mainly via GHRH and/or somatostatin modulation (1,3). The ability of a given neurotransmitter to act on either GHRH or somatostatin results in a dual mechanism of control (2–4). Different receptor subtypes can also have opposite effects. Noradrenaline (NA) has been reported to be stimulatory via \( \alpha_2 \)-adrenoceptors (5) and both inhibitory and stimulatory via \( \beta \)-adrenoceptors (6–8). GHRH secretion is also influenced by serotonergic and dopaminergic innervation (1).

Hypothalamic noradrenergic innervation arrives...
mainly along two pathways which form part of the medial forebrain bundle (MFB). The dorsal pathway originates in the locus coeruleus (A6) and sends fibers to the dorsal and paraventricular (PVN) nuclei of the hypothalamus. The ventral pathway which originates in the A1 cell group of the ventral medulla and the A2 cell group of the nucleus of the solitary tract, provides the major portion of the noradrenergic innervation of the hypothalamus (9,10). Immunohistochemical studies have shown that somatostatin is present in the parvo-cellular region of the PVN and the preoptic hypothalamic nucleus (11). The PVN serves as a convenient model system in which to study the hypothalamic integrative mechanisms since it plays a major role in the neuroendocrine response to stress (10).

Abnormal hypothalamic-pituitary-somatotropic function, reflected in reduced sleep associated GH release (12), episodic GH hypersecretion during the daytime (1,12) and a decreased response to pharmacological challenge (8,13) have been found in patients with major depressive disorder (13) but not in patients with dysthymia (12) and a decreased response to pharmacological challenge (8,13) have been found in patients with major depressive disorder (13) but not in patients with dysthymia (12) and a decreased response to pharmacological challenge (8,13) have been found in patients with major depressive disorder (13) but not in patients with dysthymia (12). The blunted GH response to clonidine (<5 ng/ml) seen in 5 out of 6 patients with primary major depressive disorder (13) has been suggested to result from hypactivity or dysregulation of the noradrenergic system (13,14). α2-Adrenoceptor-mediated regulation of the noradrenergic system has also been suggested to be abnormal in depression (13,14). However, the significance of the findings that support these suggestions remains uncertain and it is not clear whether other factors interfere with the hypothalamic-pituitary function in affective illness (12,15,16,17).

This paper aims to provide further insight into the relationship between dysfunction of the noradrenergic system and adrenergic receptor function especially in so far as it influences the hypothalamic neuronal control of the anterior pituitary.

**EXPERIMENTAL PROCEDURE**

Adult male Wistar rats (280–310g) were used. They were initially housed in groups of five per cage under standard laboratory conditions with a controlled 12h light-dark cycle (lights on from 6h00 to 18h00). Animals were allowed free access to water and Epol rat cubes.

DSP₄, N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (a gift from ASTRA), 60 mg/kg was injected intraperitoneally (i.p.) 10 days before experiments. Control rats were injected with saline. The MFB was lesioned in rats anaesthetized with Equithesin (1.5 ml) according to the co-ordinate system of Pellegrino et al (18) with the zero plane taken as the inter-aural line and the incisor bar 5.0 mm above the inter-aural line. The MFB was lesioned bilaterally (1 µl injected on either side) with 10 µg/µl 6-OHDA dissolved in 0.9% NaCl containing 1 mg/ml ascorbic acid. The coordinates AP = 5.6; ML = 1.6 and DV = 9.0 mm were used. Controls were injected with saline containing 1 mg/ml ascobic acid. The operated rats were then housed individually in single cages for 14 days.

Rats lesioned with DSP₄ or 6-OHDA were injected i.p. with clonidine (250 µg/kg, a gift from Boehringer-Ingelheim) or the β-adrenoceptor agonist, isoproterenol HCl (1 mg/kg, Sigma) 1h before decapitation.

A group of Equithesin (1.5 ml) anaesthetised rats were fitted with stainless steel guide cannulas using the procedure described by Paxinos and Watson (19) and the coordinates AP = -1.4; ML = 0.05 and DV = 8.1 mm. The tip of the cannula was 0.5 mm above the PVN and cannula placement was verified histologically. The operated rats were then housed individually in single cages and allowed to recover for 2 days. During this time the rats were handled regularly. On the day of the experiment, 1 µl of the α2-adrenoceptor agonist, clonidine HCl (0.1 µg/µl) dissolved in saline or saline were injected into the PVN. Animals were decapitated 15, 30, or 60 min after the injection.

Another group of rats were fitted with indwelling catheters for serial blood withdrawal (20) in addition to the cannula in the brain 0.5 mm above the PVN. The rats were handled daily and catheters were kept patent by washing with heparin saline. Two days after the operation the rats were injected with 1 µl of the β-adrenoceptor agonist, isoproterenol HCl (2.5 µg/µl, Sigma) dissolved in saline or saline via the cannula. Serial blood samples (0.7 ml) were withdrawn at 0 (prior to isoproterenol injection) and at 15, 30 and 60 min after isoproterenol injection. The withdrawn blood was replaced with the equivalent volume of heparin saline.

Decapitation and blood withdrawal occurred between 09:30 and 10:30 to minimise possible diurnal variation. This time interval corresponded to a trough in GH secretion (21). Blood was collected and centrifuged at 1000 g. Aliquots of the plasma were stored at −100°C until assayed. The brains were rapidly removed and placed on ice. The frontal cortex, hippocampus and hypothalamus were immediately dissected and stored at −100°C. In certain rats the PVN was removed by a modified micropunch technique (22) and stored at −100°C.

The monoamines NA, dopamine (DA) and serotonin (5-HT) were determined by reverse phase high performance liquid chromatography (HPLC) with electro-chemical detection as previously described (23). Plasma concentrations of GH were measured in triplicate using the rat GH radioimmunoassay kit kindly supplied by the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases (NIADDK, Torrance, CA, USA). The concentration of GH was determined in 50 or 100 µl plasma aliquots and the values expressed in terms of the reference preparation, rat GH-RP-2. The sensitivity of the GH assay was 0.025 ng/100 µl plasma. The intra- and interassay coefficients of variation were 11% and 14%, respectively.

The statistical significance of the results was determined by the Kruskal-Wallis and Mann-Whitney U tests. All results are expressed as mean ± S.E.

**RESULTS**

DSP₄ treatment significantly reduced the NA content of the hippocampus and the frontal cortex without significantly affecting the DA or 5-HT concentration. The significant reduction of NA in the hypothalamus (29%) was relatively small compared to the depletion of NA observed in the hippocampus (>95%) and frontal cortex (84%) (Table I, HPLC results have been published in 24).