Secretion of growth hormone elicited by intravenous desipramine in the conscious, unrestrained rat

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- 1 Acute intravenous administration of either clonidine (Clon) ($50 \mu\text{g kg}^{-1}$) or desipramine (DMI) (5 mg kg^{-1}) elicited a pulse of growth hormone (GH) and corticosterone secretion in conscious, unrestrained rats.
- 2 The responses to DMI were similar to those with Clon, except that the GH pulse following DMI was delayed and was not dose-dependent.
- 3 The GH response to DMI was inhibited by prior administration of idazoxan (1 mg kg^{-1}) or yohimbine (0.5 mg kg^{-1}), but not by atropine ($10 \mu\text{g kg}^{-1}$), sulpiride (5 mg kg^{-1}) or prazosin (1 mg kg^{-1}).
- 4 The corticosterone secretion following DMI was not altered by prior atropine, sulpiride or prazosin, but was augmented by idazoxan (1 mg kg^{-1}).
- 5 GH secretion was not influenced by atropine, sulpiride, prazosin or idazoxan given alone. Idazoxan or yohimbine given alone elicited significant secretion of corticosterone.
- 6 It is concluded that i.v. DMI caused an activation through indirect mechanisms of α_2 -adrenoceptors specifically involved in hypothalamic-pituitary regulation of GH release and also a distinct, independent and transient generalized activation of the pituitary-adrenal axis.

Introduction

The functional state of specific neurotransmitter receptors may be monitored by the measurement of anterior pituitary hormone secretion elicited by acutely administered agonist drugs (Muller, 1979). Plasma concentrations of growth hormone (GH), prolactin and thyroid-stimulating hormone have proved useful in monitoring the responsiveness of receptors involved in the neurohypothalamic-pituitary axis (Muller, 1989). Among the most extensively studied of specific responses is the prompt pulse of GH secretion elicited by administration of clonidine (Clon) in rats (Durand *et al.*, 1977; Eden & Modigh, 1977). The rat pituitary response of GH secretion following intravenous Clon has been firmly identified as a primary neural effect brought about by activation of hypothalamic α_2 -adrenoceptors (Krulich *et al.*, 1982) which are located post-synaptically (Eriksson *et al.*, 1982).

GH secretory responses to Clon, similar to those found in rats, have also been demonstrated in man and have been employed as probes of α -adrenoceptor functional status in disease states including endogenous depression (Checkley *et al.*, 1984). Intramuscular or oral administration of the tricyclic antidepressant desipramine (DMI) has also been shown to cause an acute rise in plasma GH in healthy human subjects, an effect inferred, via the blockade of noradrenaline uptake, to have been elicited by the action of increased endogenous neurotransmitter on α_2 -receptors (Laakmann *et al.*, 1986). It has been claimed that this response is also blunted in those diagnosed as suffering clinically significant depression of the endogenous type (Laakmann, 1980; Sawa *et al.*, 1982). Acutely administered DMI has a number of actions in addition to inhibition of noradrenaline reuptake, i.e. high-affinity binding to α_1 -adrenoceptors and muscarinic cholinergic receptors with lower affinity for 5-hydroxytryptamine (5-HT) and dopamine receptors (Hall & Ogren, 1981). In view of these effects of DMI and its reported efficacy in mimicking the effect of Clon in man, an investigation was made of the actions of DMI compared to Clon on GH secretion by rat pituitary.

The experiments were performed on conscious, unrestrained animals subjected to the minimum attainable degree of disturbance since both anaesthetics (Takahashi *et al.*, 1971) and stress (Schalch & Reichlin, 1968; Terry *et al.*, 1976) are known

to influence GH secretion in rats. These objectives were achieved by the use of animals implanted with chronic intravenous cannulae permitting both drug-dosing and blood-sampling in conscious animals with minimum perturbation. In order to assess any effects of pituitary activation, other than specific effects leading to the secretion of GH, plasma corticosterone was assayed in parallel with GH determinations.

Methods

Animals and surgery

Male Wistar rats (300–320 g) were anaesthetized with chloral hydrate (375 mg kg^{-1}) and intra-atrial cannulae implanted via the right external jugular vein by a method modified from that of Harms & Ojeda (1974). Briefly, the cannula was filled with heparinized saline, inserted 3 cm into the vein and secured by a cuff to the pectoralis major muscle. The free end of the cannula was then passed under the skin and drawn out at the back of the neck, so that the free end extended 1 cm. The wound was closed and the lumen sealed with a removable wire plug. On completion of surgery animals were housed one per cage in a controlled environment with a 12 h light : 12 h dark cycle (lights on at 08 h 00 min) and given food and water *ad lib*. The animals were deemed to have recovered from surgery when pre-operative weight was regained (3–5 days).

Drug challenge and blood sampling

Both injection of drugs and withdrawal of blood samples were performed via the cannula. One hour before use the cannula was unsealed and a length of polyethylene tubing (PE 50, i.d. 0.58 mm, o.d. 0.96 mm, Portex) sufficient to extend outside the cage was attached to the cannula. Drugs were injected, or blood samples withdrawn, by syringe attached to the Portex tubing. Blood was drawn into syringes rinsed with heparin (1000 u ml^{-1}) and transferred to polypropylene centrifuge tubes (Eppendorf tubes, 0.5 ml) for separation of plasma. Cells were resuspended in saline and returned to the animal following removal of the next sample.

Rat GH secretion is pulsatile in nature with a periodicity of about 3 h, the first peak occurring just after 'lights-on' and the first trough beginning 3 h later (Tannenbaum & Martin, 1976).

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Therefore, the first blood sample was withdrawn at 10 h 30 min followed immediately by an injection of saline or antagonist drug. A second pre-challenge sample was withdrawn at 10 h 50 min. Drug challenges were at 11 h 00 min, and 4 post-challenge samples were taken at 11 h 05 min, 11 h 20 min, 11 h 35 min, and 11 h 50 min. All blood samples were of 0.4 ml volume. animals were rested for 3 days before being challenged again.

Growth hormone and corticosterone assays

All plasma samples were stored frozen (-20°C) in Eppendorf tubes prior to assay. Rat GH was assayed by radioimmunoassay (RIA) with materials supplied by Dr A.F. Parlow and the National Institute for Diabetes and Digestive, and Kidney Diseases (NIDDK). Purified rat GH (NIADDK-rGH-1-5) was used for labelling with ^{125}I . The NIADDK reference preparation rGH-RP-2 was used for standards, and the antiserum used was NIADDK-anti-rGH-S-5. Addition of label was delayed by 2 h; this non-equilibrium protocol increased the sensitivity of the assay (Rodbard *et al.*, 1971). Separation of antibody-bound from free hormone was by addition of Protein A (Sigma Chemical Co.). The intra-assay and inter-assay variations were 6.9 and 11.6%, respectively.

Plasma corticosterone was routinely assayed by a modification of the chloroform extraction and ethanol:sulphuric acid fluorescence technique (Glick *et al.*, 1964). To a plasma sample (0.1 ml) in a polypropylene tube (Eppendorf tube 1.0 ml) was added 0.6 ml chloroform. Corticosterone was extracted by vortex mixing. The chloroform phase (0.5 ml) was removed to a similar tube containing 0.4 ml ethanol:sulphuric acid (35:65) and shaken to re-extract adrenocortical steroids and develop fluorescence. The fluorescence of the acid phase was determined at excitation wavelength 474 nm, emission 518 nm. Appropriate standards and blank were processed in parallel. The validity of the fluorimetric assay was checked against an RIA employing ^3H -labelled corticosterone, and rabbit anti-corticosterone 21-thyroglobulin serum. The fluorimetric method correlated well with the RIA ($r = 0.93$, $n = 24$), and plasma from animals 14 days post adrenalectomy showed no detectable corticosterone in the fluorimetric assay. The intra- and inter-assay variances were 8.3 and 11%, respectively, for the fluorescence method.

Analysis of data

GH concentrations were measured in ng ml^{-1} and are presented as median $\pm$ interquartile ranges (Clarke & Cooke, 1983). The data were not normally distributed and therefore non-parametric tests were used for group comparisons. Post-treatment levels were compared with the immediately pre-treatment levels by Wilcoxon's matched-pairs signed-rank test, each animal acting as its own control. For comparisons between treatments, differences of post-treatment values from baseline were calculated and peak changes (ΔGH) for each animal were plotted. Results were expressed as median values and the significance of any differences between medians was assessed by the Mann-Whitney U-test. Plasma corticosterone levels ($\mu\text{g dl}^{-1}$) were normally distributed and so these were expressed as mean value for a group of animals. Significance of differences between pre- and post-treatment corticosterone concentrations was assessed by paired Student's *t* test, while the unpaired *t* test was used for between-treatment comparisons.

Drugs

Chloral hydrate was purchased from BDH Chemicals, desipramine HCl, clonidine HCl, atropine SO_4 , and yohimbine HCl were purchased from Sigma Chemical Company. Prazosin HCl was a gift from Pfizer Ltd, idazoxan HCl was a gift from Reckitt and Colman Ltd, and sulpiride was a gift from De La Grange. Chloral hydrate (7.5%) was dissolved in saline,

and administered in a vol of 5 ml kg^{-1} i.p. All other drugs were injected i.v. in a volume of 1 ml kg^{-1} . Prazosin HCl (1 mg ml^{-1}) was dissolved in distilled water and sonicated. Sulpiride (5 mg ml^{-1}) was dissolved in distilled water, 1–2 drops of 1 N HCl added and sonicated, and the pH was adjusted to 6–8 with 1 N NaOH . All other drugs were dissolved in sterile saline (0.9%), to give the following concentrations per ml: 0.05, 0.1, 0.25, 0.5, 1.0 and 5.0 mg DMI ; 10, 25, 50, and $100 \mu\text{g Clon}$; $10 \mu\text{g atropine SO}_4$; $1 \text{ mg idazoxan HCl}$; $0.5 \text{ mg yohimbine HCl}$.

Results

Drug challenges

Cannulated rats, when administered saline vehicle at 11 h 00 min, did not show any subsequent significant increase in GH during the sampling period (Figure 1). Clonidine ($50 \mu\text{g kg}^{-1}$) elicited an increase in plasma GH, which was raised significantly over pre-drug levels at 20 and 35 min post-challenge. At 50 min post-injection GH had subsided to pre-drug values. DMI (5 mg kg^{-1}) also caused a pulse of GH secretion which differed temporally from the pattern after Clon in that a significant rise was achieved only at 35 min following drug challenge, but was similar in that the effect was essentially terminated 50 min post-injection.

Dose-dependence of growth hormone responses

The dependence of the GH secretory response on dose of drug was assessed by use of various dose levels of secretagogues in the standard challenge protocol. Results of these experiments

Figure 1 Median ($\pm$ semi-interquartile range, $1/2(Q_3 - Q_1)$) growth hormone (GH) levels following i.v. saline (●) ($n = 7$), clonidine $50 \mu\text{g kg}^{-1}$, (■) ($n = 8$), or desipramine (DMI 5 mg kg^{-1}), (▲) ($n = 8$). *Significantly increased over pre-treatment value at $P < 0.05$, (Wilcoxon's matched-pairs signed-rank test).

Figure 2 Median growth hormone (GH) responses ($\pm$ interquartile range) to clonidine ($n = 9$) or saline ($n = 7$) expressed as peak Δ GH. Statistically significant differences from the control group are shown as * $P < 0.005$; ** $P < 0.001$ (Mann-Whitney U-test).

are shown in Figures 2 and 3, where the magnitude of secretory response as peak Δ GH is illustrated for the dose-ranges tested. Response to Clon was statistically significant at doses above $10 \mu\text{g kg}^{-1}$; responses showed a dose-related increase for doses up to $50 \mu\text{g kg}^{-1}$, while the median response to the $100 \mu\text{g kg}^{-1}$ dose was reduced from the $50 \mu\text{g kg}^{-1}$ level (Figure 2).

Low doses of DMI (0.05 – 0.25 mg kg^{-1}), unlike clonidine at these doses, did not elicit statistically significant GH release. Doses of 0.5 mg kg^{-1} and higher doses all yielded significant responses, although no clear dose-response relationship was observed (Figure 3).

Figure 3 Median growth hormone (GH) responses ($\pm$ interquartile range) to saline or to desipramine (DMI, $n = 12$ for 5 mg kg^{-1} group, $n = 8$ for other doses), expressed as peak Δ GH. *Significantly different from saline controls at $P < 0.05$ (Mann-Whitney U-test).

Effect of receptor antagonists on the response to desipramine

Cannulated rats were given doses, chosen for optimum efficacy, of various receptor antagonists at 30 min prior to a DMI challenge performed as described above. The dose of DMI was 5 mg kg^{-1} , this being the most consistently effective of the doses of DMI employed. The influence of prior treatment with muscarinic, D_2 -dopamine receptor and α_1 - or α_2 -adrenoceptor antagonists on the subsequent response to a standard DMI challenge was investigated. Both idazoxan (1 mg kg^{-1}) and yohimbine (0.5 mg kg^{-1}) significantly reduced the GH response to subsequent challenge with DMI ($P < 0.05$ Mann-Whitney U-test). Atropine, sulpiride or prazosin did not attenuate the response. Administered alone at the same doses as used in pretreatment, none of the receptor antagonists had any detectable effect on plasma GH.

Effects of receptor antagonists on plasma corticosterone

Plasma corticosterone concentrations were determined in parallel with plasma GH following the various drug challenges investigated. Animals given saline alone showed no subsequent alteration in plasma corticosterone for the duration of the sampling period. Clon at 10 or $25 \mu\text{g kg}^{-1}$ had no significant effect; at 50 or $100 \mu\text{g kg}^{-1}$ Clon, corticosterone was elevated at 5 min post-injection. DMI at 5 mg kg^{-1} characteristically caused a significant rise in corticosterone levels also evident at 5 min post-injection. In rats treated with idazoxan prior to DMI there was an elevation in corticosterone levels compared to saline controls, but the effect was temporally different from that seen after DMI alone, being manifest at 20 min and sustained until 35 min post-injection (Figure 4).

Idazoxan alone elevated corticosterone at 5 min post-injection, the level falling back to base-line by 20 min. Yohimbine increased corticosterone levels at 20 and 35 min post-injection. As in the case of GH response, none of the other blockers (atropine, sulpiride or prazosin) had any significant effect on plasma corticosterone when administered alone. Animals administered these blockers prior to DMI failed to show a subsequent rise in corticosterone levels, although the concentrations found were not significantly lower than those obtained with DMI alone.

Discussion

The present investigation employed the technique of permanent cannulation of the jugular in order to examine neuroendocrine responsiveness in unanaesthetized, unrestrained,

Figure 4 Corticosterone levels (mean with s.e.mean shown by vertical bar) following saline ($\bullet$) ($n = 7$), desipramine (DMI, 5 mg kg^{-1}) ($\blacktriangle$) ($n = 40$), or idazoxan 1 mg kg^{-1} 30 min prior to DMI ($n = 6$). *Significantly different from pretreatment values at $P < 0.05$ (paired Student's t test).

and by implication, unperturbed animals. By the criterion of weight regain animals had recovered from the cannulation surgery within 3–5 days, and the basal levels of both corticosterone and GH, against which drug challenges were evaluated, showed that the period of day chosen was appropriate for the demonstration of subtle responses of the neurohypothalamic axis.

The GH response to intravenous Clon was very similar to that reported by Krulich *et al.* (1982). While a degree of dose-dependence was found there was a reduction noted at the highest concentration. Eden & Modigh (1977), using doses higher than $100 \mu\text{g kg}^{-1}$, failed to show a dose-response relationship. A falling-off in response may be ascribed to the possibility of α_1 -adrenoceptor effects or partial agonist behaviour of Clon at high doses. In the case of DMI, although the GH response was qualitatively similar to that evoked by Clon, two differences were apparent. Firstly, the DMI action was delayed compared to that of Clon. If, as is likely, DMI works indirectly through an increased availability of endogenous transmitter following on inhibition of noradrenaline reuptake, then some delay in onset of action may be expected even after acute intravenous administration of DMI. The second difference in the case of DMI effects was the absence of any clear dose-response relationship. This also may be explained by the indirect nature of the action of DMI on α_2 -adrenoceptors.

The response to DMI, although apparently indirectly mediated, was unequivocally blocked by prior treatment with α_2 -adrenoceptor antagonists and therefore it may be inferred that the DMI effect is brought about by a final mechanism similar to that with Clon, i.e. activation of α_2 -adrenoceptors

involved in neuro-hypothalamic regulation. The sensitivity of the GH secretion by DMI to blockade by prior idazoxan or yohimbine and the ineffectiveness of muscarinic, D_2 -dopamine or α_1 -adrenoceptor antagonists are similar to the findings on GH response to DMI in man (Laakmann *et al.*, 1986); in the rat the GH response to Clon has been shown to be also specifically blocked by α_2 -antagonist pretreatment (Eriksson *et al.*, 1982). Other theoretically possible actions of DMI on central receptors, including an action on α_1 -adrenoceptors, are not likely to be involved in the GH response. Although Krulich *et al.* (1982) found that α_1 -adrenoceptor agonists evoked a prazosin-sensitive lowering of rat plasma GH, in our experiments no effects of DMI consistent with such actions were detected.

The secretion of GH in response to DMI and to Clon was preceded by a brief pulse of corticosterone, indicating that these injected drugs activated the hypothalamic-pituitary-adrenal axis in addition to specifically triggering mechanisms leading to pituitary GH release. The results of prior administration of various blocking agents on the GH and corticosterone responses to DMI showed that the two effects were separable, since both idazoxan and yohimbine reduced the GH response but not the corticosterone secretion following DMI. In contrast, the three blocking agents which failed to alter the GH response had no clear effect on the corticosterone levels following DMI.

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References

- CHECKLEY, S.A., GLASS, I.B., THOMPSON, C., CORN, T. & ROBINSON, P. (1984). The growth hormone response in endogenous as compared to reactive depression. *Psychological Med.*, **14**, 773–774.
- CLARKE, G.M. & COOKE, D. (1983). *A Basic Course in Statistics* 2nd ed. London: Edward Arnold.
- DURAND, D., MARTIN, J.B. & BRAZEAU, P. (1977). Evidence for a role of alpha-adrenergic mechanisms in regulation of episodic growth hormone secretion in the rat. *Endocrinology*, **100**, 722–728.
- EDEN, S. & MODIGH, K. (1977). Effects of apomorphine and clonidine on rat plasma growth hormone after pretreatment with reserpine and electroconvulsive shocks. *Brain Res.*, **129**, 379–384.
- ERIKSSON, E., EDEN, S. & MODIGH, K. (1982). Up- and down-regulation of central postsynaptic alpha-2 receptors reflected in growth hormone response to clonidine in the reserpine-pretreated rat. *Psychopharmacol.*, **77**, 327–331.
- GLICK, D., VON REDLICH, D. & LEVINE, S. (1964). Fluorometric determination of corticosterone and cortisol in 0.02–0.05 milliliters of plasma or submilligram samples of adrenal tissue. *Endocrinology*, **74**, 653–655.
- HALL, H. & OGREN, S.O. (1981). Effects of antidepressant drugs on different receptors in the rat brain. *Eur. J. Pharmacol.*, **70**, 393–407.
- HARMS, P.G. & OJEDA, S.R. (1974). A rapid and simple procedure for chronic cannulation of the rat jugular vein. *J. Appl. Physiol.*, **36**, 391–392.
- KRULICH, L., MAYFIELD, M.A., STEELE, M.K., McMILLAN, B.A., MCCANN, S.M. & KOENIG, J.I. (1982). Differential effects of pharmacological manipulations of central alpha-1 and alpha-2 adrenergic receptors on the secretion of thyrotropin and growth hormone in male rats. *Endocrinology*, **110**, 796–804.
- LAAKMANN, G. (1980). Neuroendocrinological findings in affective disorders after administration of antidepressants. *Adv. Biol. Psychiat.*, **5**, 67–84.
- LAAKMANN, G., ZYGAN, K., SCHOEN, H.-W., WEISS, A., WITTMANN, M., MEISSNER, R. & BLASCHKE, D. (1986). Effect of receptor blockers (methysergide, propranolol, phentolamine, yohimbine and prazosin) on desimipramine-induced pituitary hormone stimulation in humans – I. Growth Hormone. *Psychoneuroendocrinology*, **11**, 447–461.
- MULLER, E.E. (1979). The control of somatotropin secretion. *Horm. Protein Peptides*, **7**, 123–203.
- MULLER, E.E. (1989). Some aspects of the neurotransmitter control of anterior pituitary function. *Pharmacol. Res.*, **21**, 75–85.
- RODBARD, D., RUDER, H.J., VAITURAITIS, J. & JACOBS, H.S. (1971). Mathematical analysis of kinetics of radioligand assays: Improved sensitivity obtained by delayed addition of labelled ligand. *J. Clin. Endocrinol. Metab.*, **33**, 343–355.
- SAWA, Y., ODO, S. & NAKASAWA, T. (1982). Growth hormone secretion by tricyclic and non-tricyclic antidepressants in healthy volunteers and depressives. In *New Vistas in Depression*, *Adv. Biosci.* ed. Langer, S.Z., Takahashi, T., Segawa, T. & Briley, M. Vol. 40, pp. 309–315. New York: Pergamon Press.
- SCHALCH, D.S. & REICHLIN, S. (1968). Stress and growth hormone release. In *Growth Hormone*. ed. Pecile, A. & Muller, E.E. pp. 211–225. Amsterdam: Excerpta Medica.
- TAKAHASHI, A., DAUGHADAY, W.H. & KIPNIS, D.M. (1971). Regulation of immunoreactive growth hormone secretion in male rats. *Endocrinology*, **88**, 909–917.
- TANNENBAUM, G.S. & MARTIN, J.B. (1976). Evidence for an ultradian rhythm governing growth hormone secretion in the rat. *Endocrinology*, **98**, 562–570.
- TERRY, L.C., WILLOUGHBY, J.O., BRAZEAU, P. & MARTIN, J.B. (1976). Antiserum to somatostatin prevents stress-induced inhibition of growth hormone secretion in the rat. *Science*, **192**, 565–567.

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