Alpha Adrenergic Control of Growth Hormone in Adult Male Rats

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Summary. Intravenous injection of 0.1 mg/kg clonidine into rats under urethane anaesthesia induced a prompt and long-lasting release of growth hormone, estimated by radioimmunoassay (IRGH), which could be abolished by 0.2 mg/kg phentolamine given into the 3rd ventricle. Injection of 3 μg/kg clonidine into the 3rd ventricle stimulated also the release of IRGH significantly. Intravenous administration of 0.32 mg/kg phenylephrine caused a small and transient release of IRGH only. These results provide evidence that central α-adrenergic stimulation resulting in an increased GH secretion is one important mechanism in the regulation of this hormone in the rat.

It is well established that monamines participate in the hypothalamic control of growth hormone (GH) secretion in man (Blackard and Heidingsfelder[1], Boyd et al.[2], Smythe and Lazarus[3], Lal et al.[4]), baboons (Gale and Toivola[5], Toivola and Gale[6]), rhesus monkeys (Jacoby et al.[7], Brown and Chambers[8]), dogs (Ganong et al.[9]) and rats (Müller et al.[10–14], Collu et al.[15], Kato et al.[16]).

Müller et al.[10,11], using the tibial plate bioassay for GH determination, demonstrated that drugs which interfere with central adrenergic mechanisms can block insulin-induced release of GH in the rat. Administration of norepinephrine (NE) or dopamine (DA) into the lateral cerebral ventricle of normal and hypophysectomized rats was followed by decreases in GH levels in the anterior pituitary and depletion of hypothalamic GH releasing factor Müller et al.[12,13].

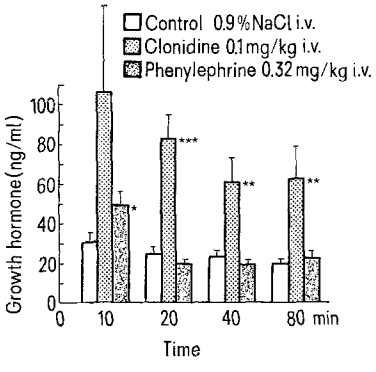

Fig. 1. Serum IRGH following i.v. administration of clonidine and phenylephrine. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ vs control. Values represent mean ± SEM of 10 to 11 animals for each group.

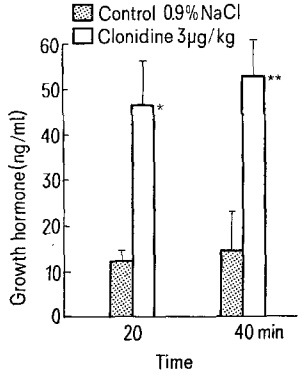

Fig. 2. Effect of clonidine injected into the 3rd ventricle on serum IRGH. $*p < 0.05$; $**p < 0.005$ vs control. Values represent mean ± SEM of 18 to 20 animals for each group.

Following the introduction of radioimmunological methods for GH determination, discrepancies were found to occur between results obtained by bioassay and those obtained with radioimmunoassay. Collu et al.[15] found that, in the rat, intraventricular administration of DA induced a decrease in plasma immunoreactive GH (IRGH), whereas central injection of NE had no effect.

Later, Kato et al.[16] reported increases of plasma IRGH in rats after systemic administration of phentolamine and chlorpromazine but also after the sympathomimetic agent methylamphetamine. Müller et al.[14] demonstrated that administration of FLA 63, a dopaminehydroxylase inhibitor as well as L-dopa into α-methyltyrosine treated rats lowered plasma IRGH. This led him to postulate that GH secretion is under a dual aminergic control; an α-adrenergic component which stimulates and a dopaminergic component which inhibits the secretion of GH.

We have examined the effects of clonidine, a centrally acting α-adrenoceptor stimulant drug (Schmitt et al.[17]) and phentolamine, an α-adrenoceptor blocking agent, in order to clarify the role which central α-adrenoceptors play in the control of GH secretion in the rat.

Material and methods. Adult Sprague-Dawley male rats (200–240 g) were used. The animals were kept under standard conditions, i.e. 14 h light (from 04.00 h–18.00 h),

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Effect of phentolamine injected into the 3rd ventricle on the clonidine induced IRGH release.

Drugs or solvent	IRGH ng/cm³
Control (0.9% NaCl i.v.)	24.8 ± 6.7
Clonidine (0.1 mg/kg i.v.)	58.0 ± 12.7[a]
Phentolamine into the 3rd ventricle (0.2 mg/kg)	17.1 ± 2.6
Phentolamine into the 3rd ventricle (0.2 mg/kg) + clonidine (0.1 mg/kg i.v.)	21.2 ± 6.9[b]

[a] $p < 0.05$ vs. control; [b] $p < 0.05$ vs. clonidine. Phentolamine was given 10 min prior to the clonidine administration. Serum IRGH was determined 40 min after the clonidine administration. Values represent mean ± SEM of 16 to 21 animals in each group.

24 °C. Food was removed on the evening preceding the experiment. On the day of the experiment the animals were anesthetized with urethane (1.35 g/kg i.p.); 45 min later, the test substances were injected into a jugular vein in a volume of 0.2 cm³/100 g bodyweight.

For injection into the 3rd ventricle, the anesthetized rats were placed in a stereotaxic apparatus 2 min before injection. A small hole, approximately 1 mm in diameter was drilled behind the bregma and a cannula of 0.5 mm outer diameter lowered into the 3rd ventricle at its junction with the lateral ventricles using the coordinates of DE GROOT's atlas[18]. The drugs or solvent were injected in a volume of 10 µl using a microsyringe. 10, 20, 40 and 80 min after i.v. or intraventricular injections, the animals were decapitated and serum samples were collected for GH determination. The serum was kept frozen at −20 °C until assayed. Serum IRGH was measured in triplicate with double antibody radioimmunoassay using NIAMD RGH-RP-1 as standard[19].

The following drugs were employed: clonidine HCl, phentolamine HCl and phenylephrine HCl. Data were evaluated statistically with Student's *t*-test.

Results. Figure 1 shows the time pattern of IRGH release induced by 0.1 mg/kg clonidine. The release was immediate (10 min) and long lasting. Plasma levels were still significantly elevated 80 min after the injection ($p < 0.01$). Intravenous injection of 0.32 mg/kg phenylephrine (Figure 1) elicited only a short lasting small increase of serum IRGH 10 min after its administration ($p < 0.05$).

Figure 2 demonstrates the effect of administration of 3 µg/kg clonidine into the 3rd ventricle; 20 and 40 min after the injection, serum IRGH was significantly elevated ($p < 0.05$; $p < 0.005$ respectively).

GH release induced by 0.1 mg/kg i.v. clonidine, measured 40 min after its administration was completely abolished by pretreatment with 0.2 mg/kg phentolamine into the 3rd ventricle 10 min prior to the clonidine injection (Table).

Discussion. Clonidine is a potent α-adrenoceptor stimulant both in the central nervous system and in the periphery (KOBINGER and WALLAND[20], STRUYKER BOUDIER et al.[21]). It has been shown that it can influence several centrally regulated autonomic functions, such as blood pressure (KOBINGER and WALLAND[20], body temperature (MASKREY et al.[22]), food intake (BROEKKAMP and VAN ROSSUM[23]) blood glucose (BOCK and ZWIETEN[24]) and ACTH secretion (GANONG[9]).

Our findings clearly demonstrate that clonidine stimulates the release of IRGH. This effect could be abolished completely by central administration of phentolamine, suggesting that central α-adrenoceptors are concerned in IRGH release (Table). Further support for a central mechanism was provided by the fact that IRGH stimulation occurred in response to 3 µg/kg clonidine, when injected into the 3rd ventricle (Figure 2), whereas the same dose given systematically elicited a non-significant IRGH release only.

It is unlikely that clonidine stimulates IRGH secretion by other mechanisms. Stress stimuli such as insulin-induced hypoglycemia, inhalation of ether (SCHALCH and REICHLIN[25]), cold exposure (EISENBERG et al.[26]), histamine and formalin stress (KATO et al.[16]) either lower or fail to influence plasma IRGH.

In our experiments, phenylephrine, a selective α-adrenoceptor stimulant with minimal central effects (GOODMAN and GILMAN[27]) induced a small and short-lasting release of IRGH. BIRGE et al.[28] demonstrated that neither NE, DA nor phenylephrine significantly influenced the secretion of IRGH of isolated pituitaries. Since both the pituitary and the hypothalamus are to a certain extent permeable to catecholamines (AXELROD et al.[29], WILSON et al.[30]), it can be assumed that the effect of phenylephrine was due to an action at the level of the hypothalamus.

Our results are not in accordance with the results of COLLU et al.[15] who were unable to demonstrate any effect of intraventricularly administered NE on plasma IRGH in rats. One explanation for this discrepancy might be that intraventricularly injected NE is metabolized before it reaches its site of action.

KATO et al.[16] reported that systemic administration of phentolamine caused a transient release of IRGH in rats. In these experiments, systemic administration of amphetamine into rats also caused a release of IRGH (KATO et al.[16]). On the other hand, KOKKA et al.[31] demonstrated an inhibition of IRGH release in response to amphetamine.

At present it is difficult to reconcile these conflicting results. It is possible that the same drug – depending on the dosage and the experimental procedure – exerts different effects on GH secretion. Moreover, it cannot be excluded that the same neurotransmitter or drug may have opposite effects on the release of GH if it acts at different

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sites within the central nervous system. Such a possibility is supported by the recent findings of HALE and SYMINGTON[32] who studied the effects of DA on FSH release by isolated pituitaries in the presence of whole hypothalami, when release was increased, while in the presence of median eminence tissue release was decreased.

There is considerable evidence that central α-adrenoceptor stimulation induces the release of IRGH in higher species. In dogs, phentolamine, administered intraventricularly, abolished the release of IRGH induced by the NE precursor L-dopa (GANONG [9]). In conscious cats similar results were obtained with phenoxybenzamine but not with the dopamine receptor blocking agent pimozide (RUCH and GALE, unpublished observations). BROWN and CHAMBERS[8] reported that systemic administration of clonidine and DL-threodops (the immediate precursor of NE) induced an increase of IRGH in rhesus monkeys. TOIVOLA and GALE[6] demonstrated that microinjection of NE into or in the vicinity of the ventromedial nucleus of baboons induced a release of IRGH.

In humans, IRGH release following insulin hypoglycemia was attenuated by phentolamine (BLACKARD and HEIDINGSFELDER[1]). More recently, it was demonstrated that clonidine increased IRGH in human subjects (LAL et al.[4]).

Our findings provide evidence that α-adrenergic stimulation is one important mechanism in the regulation of GH secretion in the rat, as already postulated by MÜLLER et al.[14]. It seems, therefore, that the control of GH secretion through an α-adrenergic mechanism in this species is probably similar to that found in higher species, including primates.

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Chromatographic Conditions in the Expression of Corticosteroid Receptor Specificity

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Summary. It is shown that cytosol preparations bound with various concentrations of a steroid are necessary to reveal physicochemically distinct, heterogeneous and polymorphic receptors present in the hormone specific target organ, that these cannot be fully appreciated in one-shot experiments at suboptimal steroids levels, and that they escape detection by equilibrium binding and Scatchard analysis alone.

The first step in the mechanism of corticosteroid hormone action is currently believed to consist of the binding of the steroid molecule with its appropriate intracellular receptor leading, thereafter, to timely, sequential and selective genetic modulation[1]. Contrary to the usual association-dissociation studies used to reveal the presence of high affinity hormone-specific binding sites in the target tissue, in 1970 we pioneered partial purification of corticosterone binders in rat liver and demonstrated that the receptor moieties exist subdivided into physicochemically distinct subpopulations which, naturally, cannot be revealed by Scatchard analysis alone[2]. More recently, these same techniques were adapted to revealing a mineralocorticoid (aldosterone) specific receptor present only in the target tissue kidney, and absent from the liver[3]. In the present report we describe the saturation characteristics and chromatographic behaviour of these latter in comparison with the glucocorticoid receptors that appear to be identical in most tissues studied. We chose to employ DE-52 gels for this purpose since they appear to be ideally suited as compared to a number of other chromatographic procedures tested[4].

Material and method. Male, Wistar rats (150–200 g) were bilaterally adrenalectomized 2–3 days prior to sacrifice by exsanguination under light ether anaesthesia.

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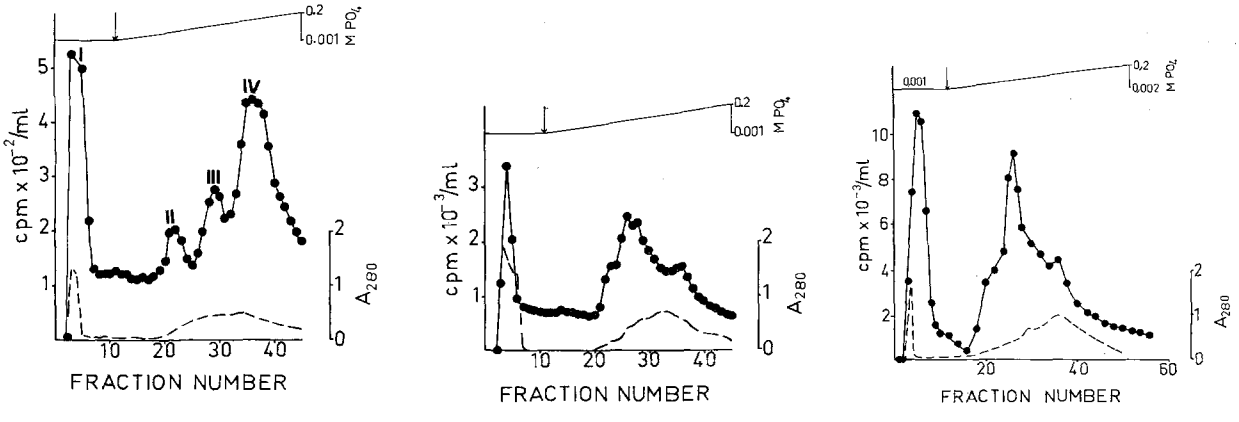

Fig. 1. Dose dependant separation of aldosterone binding proteins in rat kidney.