Effect of carbamylcholine, GTP and GTPγS on [^{3}H]inositol 1-phosphate production by prelabeled digitonized RINm5F cells

Increment in inositol 1-phosphate production (amol/10^3 cells per 10 min)	
Carbamylcholine (1.0 mM)	1.56 ± 0.56 (15)
GTP (10 μM)	1.52 ± 0.68 (9)
GTP (10 μM) + carbamylcholine (1.0 mM)	8.56 ± 1.52 (9)
GTPγS (10 μM)	9.98 ± 0.73 (9)

ence to the specific activity of the preincubation medium. As shown in the table, carbamylcholine (1.0 mM) and GTP (10 μM), when tested separately, caused a modest but significant ($p < 0.05$) increase in [^{3}H]inositol 1-phosphate production. Such a production was markedly increased in the simultaneous presence of carbamylcholine and GTP. The increment in [^{3}H]inositol 1-phosphate production recorded in the presence of both agents was much higher ($p < 0.005$) than that computed by summing their individual effects, and represented 86 ± 16% (n = 9) of the paired increment evoked by a stable analog of GTP (GTPγS). The production of [^{3}H]-inositol 1-phosphate, 1,4-bisphosphate and 1,4,5-trisphosphate was also stimulated either by carbamylcholine, in intact RINm5F cells, or by GTPγS in a subcellular particulate fraction derived from these cells (data not shown).

The present results provide direct evidence to support the participation of a GTP-binding protein in the control of phospholipase C activity in pancreatic islet cells. Since we have previously reported[7] that neither of the two regulatory proteins Ns and Ni, which were both recently identified in islet cells[8,9], seem to affect phospholipase C activity in such cells, the GTP-binding protein mediating the activation of the latter enzyme by cholinergic neurotransmitters is likely to represent a novel regulatory protein. This proposal is consistent with recent studies[10,11] indicating the participation of a) guanine nucleotide-dependent regulatory protein(s), such as the *ras*-encoded p21 protein[11], in the stimulation of phospholipase C, although none of these previous studies deal with the activation of the latter enzyme by cholinergic agents.

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Somatostatin immunoneutralization overcomes the inhibitory effects of quipazine and pargyline on growth hormone secretion in domestic fowl

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Summary. The inhibitory effects of pargyline and quipazine on chicken growth hormone secretion were overcome by passive immunoneutralization of endogenous somatostatin (SRIF)-14 or SRIF-28(1–14)-like immunoreactivity. Administration of the specific antisera to control birds pretreated with 0.9% NaCl elevated the basal plasma GH concentrations. These results suggest that peptides with SRIF-14 or SRIF-28(1–14)-like immunoreactivity tonically inhibit GH secretion and are at least partially responsible for the inhibitory effects of pargyline and quipazine on GH release in immature domestic fowl.
Key words. Somatostatin; growth hormone; serotonin.

The release of pituitary growth hormone (GH) is inhibited by hypothalamic factors, somatostatins (SRIFs), that are secreted into hypophysial blood in response to neural information concerning the internal or external environment[1]. Both somatostatin-14 and SRIF-28 have been located in the avian hypothalamus[2] and both inhibit GH secretion in birds[3]. At least two other somatostatin moieties have been located in the avian hypothalamus[4] and peptides with SRIF-28(1–14) immunoreactivity, distinct from SRIF-14 and SRIF-28, are also likely to be present, since chickens immunized against SRIF-28(1–14) have elevated plasma GH concentrations[5]. These hypophysiotrophic factors may be released in response to increased serotoninergic activity, since hypothalamic serotonin turnover is inversely related to the plasma GH level[6] and the in vitro GH releasing activity of the hypothalamus is reduced following in vivo administration of serotoninergic drugs to fowl[7,8]. Drugs that enhance serotoninergic activity, including precursors (tryptophan and 5-hydroxytryptophan), receptor agonists (e.g. quipazine), re-uptake inhibitors (e.g. imipramine) or inhibitors of serotonin degradation (e.g. pargyline and clorgyline) all reduce plasma GH concentrations in fowl, while inhibitors of serotonin synthesis (e.g. parachorophenylalanine or a deficiency of dietary tryptophan), or antagonists of serotonin receptors (e.g. methysergide and cyproheptadine) block the effects of serotoninergic drugs or increase circulating GH concentrations[6–10]. Since serotonin directly affects SRIF-14 release from the rat hypothalamus[11] and as the inhibitory effects of quipazine on hypothalamus-induced GH secretion can be suppressed, in vitro, by the addition of SRIF-14 antisera to the incubation media[12], the serotoninergic inhibition of in vivo GH secretion in birds may thus be SRIF-mediated. This possibility has been investigated in the present study, by determining the effects of somatostatin immunoneutralization on GH secretion in birds pretreated with quipazine or pargyline.

Material and methods. Specific antisera, raised in rabbits against a synthetic cylic SRIF-14-glutaradehyde-human serum albumin conjugate (product No. AB 25) and against synthetic Tyr-soma-

tostatin-28, fragment (1–14) (product No. AD 75) were obtained commercially (Cambridge Research Biochemicals, Cambridge). The SRIF-28(1–14) antiserum was specific for the C-terminus of the somatostatin 28, fragment (1–14) sequence and the cross-reactivity of SRIF-28 with this antiserum was only 1.3%. This antiserum also had no cross-reactivity with SRIF-14, gastrin 17, glucagon, gastrin 34, pancreatic polypeptide, GIP, bombesin, gastrin releasing peptide, ACTH, dynorphin, GH, FSH, LH, TSH, substance P, secretin, TRH, litorin, neurotensin, human C peptide, LPH or met-enkephalin. These antisera were administered (0.2 ml/kg i.v.) to 6-week-old white leghorn cockerels which had been pretreated 60 min earlier with quipazine maleate (Miles Research, CA; 10 mg/kg, i.p.), pargyline hydrochloride (Sigma, Poole, Dorset; 80 mg/kg, i.p.) or the 0.9% NaCl vehicle (1.0 ml/kg, i.p.). Control birds received serum from non-immunized rabbits (NRS). Heparinized venous blood samples were collected from each bird before and after the administration of these drugs and following antiserum administration. Plasma was stored at − 20 °C prior to analysis for GH content, by homologous radio-immunoassay (Harvey and Scanes, 1977). Statistical differences in the results were determined by paired and unpaired Student's t-test.

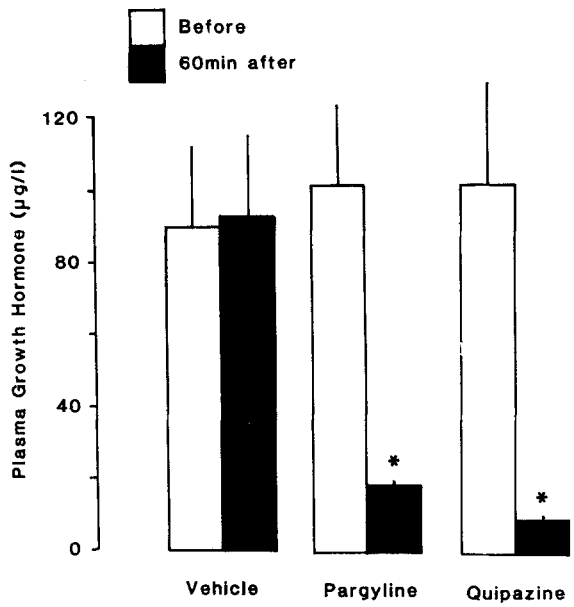

Figure 1. Concentrations of growth hormone in the plasma of 6-week-old cockerels before and 60 min after the i.p. administration of pargyline HCl (80 mg/kg), quipazine maleate (10 mg/kg) or the 0.9% NaCl vehicle (1.0 ml/kg). Means ± SEM (n = 18). The asterisks indicate significant differences from the pretreatment level ($p < 0.001$).

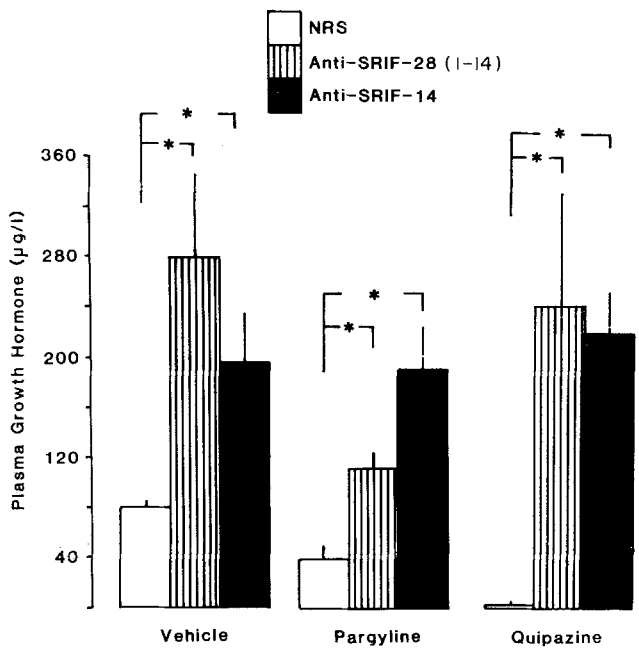

Figure 2. Concentrations of growth hormone in the plasma of 6-week-old cockerels 20 min after the i.v. injection of rabbit-anti-SRIF-28(1–14) (shaded bars), rabbit-anti-SRIF-14 (filled bars) or serum from non-immunized rabbits (NRS, open bars). 60 min before serum administration the birds were injected i.p. with pargyline HCl (80 mg/kg), quipazine maleate (10 mg/kg) or the 0.9% NaCl vehicle (1.0 ml/kg). Means ± SEM (n = 6). Significant differences between groups are indicated by the asterisks ($p < 0.05$).

Results and discussion. The plasma GH concentration was unaffected by the administration of 0.9% NaCl, but was markedly reduced ($p < 0.001$) 60 min after the injection of pargyline or quipazine (fig. 1). The GH concentration following quipazine administration was lower ($p < 0.01$) than that in the pargyline-injected birds. These results confirm previous findings and demonstrate the inhibitory effect of the serotoninergic system on GH secretion in fowl[6–8].

The inhibitory effects of pargyline and quipazine on the plasma GH concentration were still observed 20 min later, after the administration of NRS (fig. 2). While NRS had no affect on the GH concentration in each group, both anti-SRIF-14 and anti-SRIF-28(1–14) consistently increased ($p < 0.05$) the GH concentration. The magnitude of the GH response to anti-SRIF-14 was similar in each group. The GH response of the pargyline-pretreated birds to anti-SRIF-28(1–14) was less ($p < 0.05$) than that in the controls and less ($p < 0.05$) than the GH response of the pargyline-treated birds to anti-SRIF-14 administration. These results therefore suggest that peptides with SRIF-28(1–14) and SRIF-14-like immunoreactivity are at least partially responsible for the inhibitory effects of serotonin on GH secretion.

Growth hormone release in immature birds would appear to be under tonic SRIF inhibition, since anti-SRIF-14 and anti-SRIF-28(1–14) both increased the GH concentration in the vehicle-injected birds, in agreement with previous findings[5,13]. The prompt increase in the GH concentrations following SRIF immunoneutralization probably occurs in response to endogenous GH-releasing factors, and is similar to the 'rebound' in GH secretion following the withdrawal of SRIF-14 or SRIF-28 infusion[3].

Since specific immunoneutralization of endogenous SRIF-14 or SRIF-28(1–14)-like immunoreactivity overcomes the inhibitory effect of pargyline and quipazine on GH secretion, peptides with SRIF-14 and SRIF-28(1–14)-like immunoreactivity are likely to mediate the inhibitory effects of these drugs. This suppression of GH secretion is unlikely to result from a decrease in hypothalamic stimulation, as increased plasma GH levels in response to SRIF immunoneutralization would then be unlikely to occur, especially as very little GH is released autonomously[14]. Moreover, although the GH response to anti-SRIF-28(1–14) was reduced in the pargyline pretreated birds in comparison with the controls, this is unlikely to reflect reduced hypothalamic stimulation as the GH response following SRIF-14 immunoneutralization was unchanged. The reduction in the GH response to anti-SRIF-28(1–14) in the pargyline pretreated birds is therefore likely to be due to a decrease in the rate of release of the peptide with SRIF-28(1–14)-like immunoreactivity. Different SRIF peptides can be differentially released from hypothalamic neurones in response to provocative stimuli[15], and the inhibitory effect of pargyline on GH secretion is probably mediated by a relative increase in SRIF-14 release.

In summary, these results demonstrate that SRIF immunoneutralization stimulates GH secretion and also overcomes the inhibitory effects of pargyline and quipazine on GH secretion in fowl.

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Potentiating effect of aldosterone on the diuretic action of atrial extract

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Summary. The typical stimulatory effect of a rat heart atrial extract on urinary water, sodium, potassium and kallikrein excretion is significantly increased by a previous administration of aldosterone (0.5 µg/100 g b. wt) in the rat.
Key words. Atrial natriuretic factor; aldosterone; urinary kallikrein; natriuresis; kidney.

Crude extracts of mammalian cardiac atria have powerful natriuretic and vascular muscle relaxant activity[1,2]. These effects are mediated by peptides identified as atrial natriuretic factors (ANF). All contain the same core sequence of 17 amino acids but differ in the lengths of their amino and carboxyl termini[3]. Circulating ANF have been detected in the blood by radioimmunoassay, suggesting that they have an endocrine role in fluid and electrolyte homeostasis[4-7]. One striking feature of ANF is its ability to inhibit the secretion of aldosterone, the most potent physiological factor promoting sodium reabsorption by the kidneys. ANF reduces basal secretion of aldosterone in vivo[8] and the secretion induced by angiotensin stimulation of the adrenal cortex in vitro[9,10]. Suppression of aldosterone secretion in vivo is amplified by the concurrent inhibition of renin secretion, the enzyme which liberates angiotensin[11]. The accumulated evidence leads to the cogent assumption that a rise of aldosterone concentration in the blood by exogenous administration may hamper ANF-induced natriuresis. But contrary to this expectation, our results show that natriuresis is significantly enhanced by administration of a moderate dose of aldosterone 4 h before an i.v. bolus of a standard dose of ANF.

In the present experiments, the urinary excretory rates of water, sodium, potassium and kallikrein were compared in two groups of rats (8 in each). Both groups were given the same dose (0.1 ml) of rat cardiac atria extract containing semi-purified ANF. One group, 200–220 min before the administration of ANF, received 0.5 µg/100 g b. wt i.p. of d-aldosterone (Sigma) diluted in isotonic glucose solution (0.1 ml); the other one only the vehicle. According to previous reports the effects of exogenous aldosterone can be registered within a few hours (2–4 h). The dose of this mineralocorticoid employed here is close to the physiological range[12].

Female Sprague-Dawley rats (200–220 g) were used. They were fed with the normal laboratory diet, which contains 180 mg of Na and 750 mg of K/100 g, and had tap water ad libitum. 12 h before the experiment no food was given. The animals were kept at constant temperature with a 12-h alternated rhythm of light and darkness, they were gently manipulated and any stressing factor (noise, seclusion, etc.) was prevented. According to our experience, prevention of any type of stress during the manipulation of the rat significantly reduces the variability in the bioassay, which is particularly important for the assessment of the natriuretic activity[13]. Both groups, those injected with aldosterone and the controls injected with the vehicle, were anesthetized 1 h later by i.p. injection of sodium penthotal (4 mg/100 g b.wt). The rats were then returned to the cages, and (while deeply anesthetized) transported to the place where the surgical procedure was carried out. Blood pressure was continuously recorded through a cannula placed in the left carotid artery; a catheter introduced in the bladder through the urethra allowed continuous collection of urine.

Urine volume, the amounts of sodium and potassium, and kallikrein activity, excreted every 20 min, were measured for a period of 200 min. Throughout the experiment the rats were infused with 0.60 ml/100 g b.wt/60 min of isotonic glucose solution into the jugular vein. The amounts of sodium and potassium excreted, measured by a flame photometer (Eppendorf) were expressed in µmol per 100 g b.wt excreted in 20 min. Kallikrein activity was determined by the amidase method[14] and expressed as nmol of p-nitroaniline generated per min at 37 °C. The urinary excretion of kallikrein was calculated by the urine volume and referred to 100 g b.wt. The determination of kallikrein was included in this study because several lines of investigation suggest a role for the renal kallikrein-kinin system in the regulation of renal hemodynamics, fluid and electrolyte homeostasis[15].

The ANF preparation was obtained as folllows: atria were excised from 300 adult rats. The tissue was rinsed with cold saline, homogenized, mixed with 5 volumes of cold 0.1 M acetic acid, and centrifuged. The supernatant was heated in a boiling water bath for 15 min. After cooling, the precipitate was removed by centrifugation and the supernatant neutralized with ammonia. To preclude the action of proteases, 1 mg of ovomucoid and 1 mg of soy-bean antitrypsin inhibitor were added. After a final centrifugation the extract was lyophilized. This material was dissolved in saline (45 ml) and stored in vials at − 40 °C. The