these secretion rates could not be correlated to corticosteroid plasma concentrations ($r = 0.14$, $p > 0.05$) or adrenal weights ($r = 0.11$, $p > 0.05$). The importance of the findings presented here is further underlined by our very recent work which showed a positive correlation between basal and (1–24)ACTH-stimulated corticosteroid (table 3) and aldosterone/progesterone[22] secretion by the adrenal gland of the tree shrew *(Tupaia belangeri)* superfused in vitro. Similarly to the findings in the Mongolian gerbil, in vitro secretion of steroids by the tree shrew adrenal gland was not correlated either to corresponding plasma levels ($r = -0.41$, $p > 0.05$) or to adrenal weights ($r = 0.12$, $p > 0.05$)[22].

To summarize, superfusion of adrenocortical tissue from individual animals gives insights into aspects of adrenal function which cannot be investigated by static incubation of tissue blocks or isolated cells. The significant positive correlation between basal secretion and the responsiveness to (1–24)ACTH indicates that the fine adjustment of basal corticosteroidogenesis (also[11, 13, 23]) forms an important part of the regulatory mechanisms modulating (1–24)ACTH- stimulated corticosteroid secretion in vitro.

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Anomeric specificity of glucose-induced somatostatin secretion

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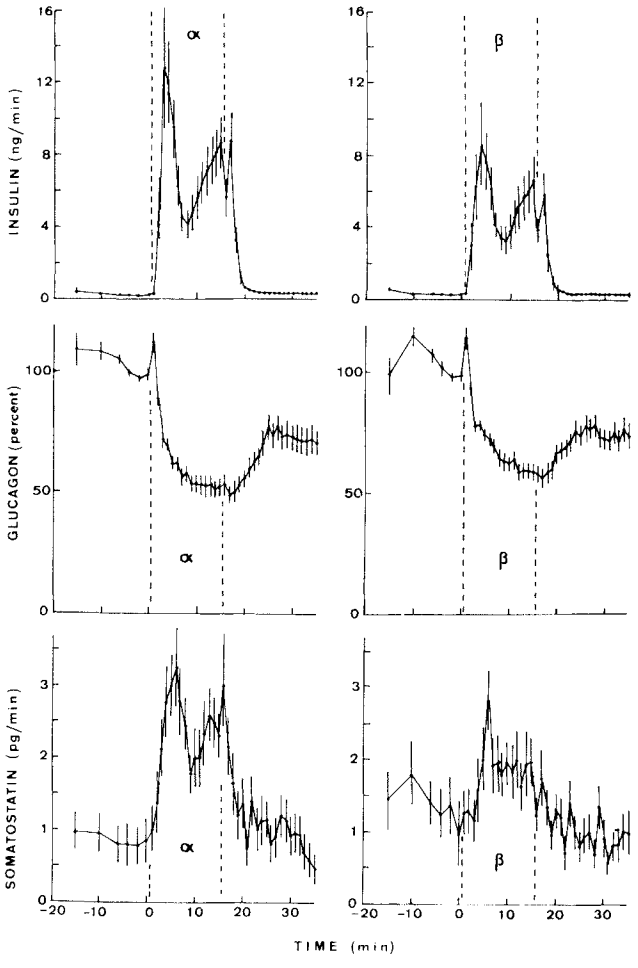
Summary. In isolated perfused rat pancreases, the α-anomer of D-glucose is more potent that β-D-glucose not solely in stimulating insulin release and suppressing glucagon output, but also in causing somatostatin secretion.
Key words. D-glucose anomers; endocrine pancreas; insulin; glucagon; somatostatin.

Several functional processes triggered by D-glucose (or D-mannose) display anomeric specificity. For instance, α-D-glucose is better able than β-D-glucose to evoke the taste of sweetness[1], to stimulate insulin release[2] and to inhibit glucagon secretion[3]. Likewise, in several cell types, including pancreatic islet cells[4–6], erythrocytes[7], adipocytes[8], hepatocytes[9] and brain cells[10], the metabolism of α-D-glucose differs from that of β-D-glucose. The latter difference persists in cells exposed to equilibrated D-glucose[8, 10]. The anomeric specificity of D-glucose metabolism is thought to result from adaptation to the anomeric environment, which is itself imposed by a thermodynamic constraint[11, 12]. The anomeric specificity of enzymes indeed underwent a phylogenetic evolution[13] and participates in the fine control of D-glucose catabolism[5, 12]. Moreover, the anomeric preference of functional processes is apparently perturbed in certain pathological situations[1, 14, 15]. These considerations justify, in our opinion, further efforts to assess the existence, mechanism and significance of such an anomeric specificity in distinct cell types. In this respect, the present report reveals that α-D-glucose stimulates more efficiently than β-D-glucose the secretion of somatostatin from the perfused rat pancreas.

Female Zucker rats (13–26 weeks old), fed ad libitum, were anesthetized with sodium barbital (42 mg/kg, i.p.), and the pancreas isolated from all adjacent organs and perfused through the celiac and superior mesenteric arteries[16]. The basal medium consisted of a Krebs-bicarbonate-buffer[17] containing L-leucine (10 mM), dextran T 70 (40 g/l) and bovine albumin (5 g/l). The anomers of D-glucose were dissolved in chilled saline and administered through a side-arm syringe maintained under ice. The flow rate was fixed at 1.6 ml/min. The methods used for the immunoassay of insulin, glucagon and somatostatin were previously described[17].

In each experiment, the α- and β-anomers of D-glucose (final concentration: 3.3 mM) were each administered twice for 15 min, followed by a 20-min period of glucose deprivation. The experiments were performed in 6 lean (192 ± 9 g b.wt) and 6 obese (284 ± 19 g) Zucker rats. In each type of rat, 3 animals received the D-glucose anomers in the sequence α1-β1-α2-β2 (α1 refers to the first administration of α-D-glucose, and α2 to its second administration) and the other 3 rats in the opposite order (β1-α1-β2-α2). No significant difference between lean and obese rats was found for the pancreas wet weight (0.64 ± 0.03 g), its somatostatin content (643 ± 39 ng/g), the basal somatostatin output (1.7 ± 0.6 pg/min) and the integrated somatostatin release during the entire experiment (238 ± 38 pg or, relative to the pancreas content, 0.06 ± 0.01%), the values quoted in parentheses representing pooled data (lean and obese; mean ± SEM; n = 11–12). The data for somatostatin release collected for one lean rat in



Time course for insulin, glucagon and somatostatin output in response to the administration of α- and β-D-glucose. Mean values (± SEM) refer to the 22–24 individual determinations, and are expressed either in absolute terms (insulin and somatostatin output) or as a percentage of the mean secretory rate recorded, in the same experiment, over the last 4 min just prior to introduction of the hexose (glucagon output).

the β1-α1-β2-α2 series could not be used because the hormonal release remained undetectable (< 0.1 pg/min) throughout the experiment. Likewise, the data for glucagon release collected for another lean rat of the same series were discarded because, in this animal, α-D-glucose failed on two successive occasions to affect glucagon output.

The mean rate of somatostatin release during the administration of α- and β-D-glucose is illustrated in the figure, together with the corresponding values for insulin and glucagon secretion. The results suggest that α-D-glucose increased the release of somatostatin more than β-D-glucose. The interpretation of secretory data needs, however, to take into account the priming action of D-glucose upon hormonal output, as resulting from the repeated administration of the hexose[18]. For instance, the mean value for the paired α2/α1 and β2/β1 ratios in somatostatin release averaged 1.65 ± 0.26 (n = 22), which is significantly different from unity ($p < 0.05$) and indicates that somatostatin secretion occurred at a higher rate during the second than during the first administration of each anomer. In order to correct for such a priming action, the α1/β1 and α2/β2 ratios in hormonal secretion, as recorded in the α1-β1-α2-β2 series, were compared, respectively, to the β1/α1 and β2/α2 ratios established in the β1-α1-β2-α2 series. The results collected in the latter series were eventually expressed relative to the mean

Anomeric ratio in insulin, glucagon and somatostatin secretion

Series Ratios	α1-β1-α2-β2 α1/β1 and α2/β2	β1-α1-β2-α2 β1/α1 and β2/α2	p
Insulin	100.0 ± 6.0 (12)*	38.6 ± 4.3 (12)	< 0.001
Glucagon	100.0 ± 10.7 (12)	48.9 ± 5.6 (10)	< 0.001
Somatostatin	100.0 ± 11.5 (12)	56.9 ± 9.4 (10)	< 0.020

* The anomeric ratio for the stimulatory action of D-glucose upon insulin and somatostatin release and its inhibitory action upon glucagon secretion was established at identical times in the α1-β1-α2-β2 and β1-α1-β2-α2 series. The results obtained in the latter series were expressed as a percentage of the mean corresponding value recorded for the same type of animal in the former series. Mean values (± SEM) are shown together with the number of individual ratios (*in parentheses*) and statistical significance (p) of anomeric differences.

value recorded, at the same time and in the same type of animals (lean or obese), in the former series. As indicated in the table, the secretory response to β-D-glucose represented only about half of that evoked by α-D-glucose. In this comparison, the secretory response to each anomer was judged either from the integrated insulin and somatostatin output during the entire period of exposure to each anomer or from the percent fall in glucagon secretion observed 4–6 min after introduction of D-glucose. Incidentally, although the anomeric difference in glucagon output appeared somewhat more marked during the early than during the late period of exposure to D-glucose, it nevertheless still achieved statistical significance at the end of such a period (min 13 to min 15). None of the parameters documenting the anomeric specificity of the secretory response to D-glucose was significantly different in lean and obese animals, respectively, the results obtained in these two types of rats being eventually pooled together for the sake of simplicity in presentation.

In conclusion, the present results reveal that α-D-glucose is more potent than β-D-glucose not solely in stimulating insulin release and suppressing glucagon output, but also in causing somatostatin secretion from the perfused rat pancreas. The identical anomeric specificity of this triple secretory response is compatible with the view that the metabolic regulation of hormonal release in the endocrine pancreas reflects a coordinated behavior of all islet cells[19], possibly mediated by direct cell-to-cell communication through the network of gap junctions[20,21].

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4-Proline and 4-hydroxyproline analogs of arginine vasopressin: Role of the proline substitution in the two β-turns of vasopressin

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Summary. [4-L-Proline]arginine vasopressin, [4-D-proline]arginine vasopressin, [4-hydroxyproline]arginine vasopressin and [4-proline, 7-hydroxyproline] arginine vasopressin were synthesized and found to have antidiuretic activities of 91 ± 4, 1.7 ± 0.2, 1.0 ± 0.1 and 4.4 ± 1.0 units/mg, respectively. None of these analogs exhibited a significant level of rat pressor activity. The observed activities of these and other analogs with substitutions at position 4 and/or 7 are discussed on the basis of hypotheses and data bearing on the solution conformation of vasopressins.
Key words. Vasopressin; position 4 and 7 analogs; structure-activity analysis; solid phase peptide synthesis.

The results of solution studies by NMR spectroscopy[1,2] and solid state studies by X-ray crystallography[3] suggest that the two major structural features of oxytocin and arginine vasopressin (AVP) are two β-turns. One β-turn involves positions 2–5 (the sequence -Tyr-Phe-Gln-Asn-) and the other, positions 6–9 (the sequence -Cys-Pro-Arg-GlyNH$_2$). Proline, the amino acid which is classically inserted into a sequence in order to 'force' it into a β-turn, occurs naturally in position 7 (in the second β-turn). Hydroxyproline (Hyp), which may hydrogen bond to receptors, improves antidiuretic activity[4] but destroys pressor activity when substituted for proline in position 7. In the first β-turn, Tyr-Phe-Gln-Asn, glutamine is thought to play an important role in maintaining the β-turn structure[5]. In the present study we have sought to compare this role of glutamine with the role of proline by substituting proline and hydroxyproline, for glutamine in position 4. We here report the synthesis and biological activities of the following four compounds: [4-L-proline] arginine vasopressin, [4-D-proline] arginine vasopressin, [4-hydroxyproline] arginine vasopressin and [4-proline, 7-hydroxyproline] arginine vasopressin.

Materials and methods. Antidiuretic activities were assayed on water-loaded ethanol-anesthetized rats according to the procedures of Sawyer[6]. Pressor activities were assayed on urethane-anesthetized, phenoxybenzamine-treated rats as described by Dekanski[7].

The preparation of analogs I, II, III and IV was performed by solid phase techniques[8] according to a well established procedure used for the synthesis of many AVP analogs[9]. 1 g of 1% cross-linked benzhydrylamine-HCl resin (0.67 mequiv. amine/g of resin) was the solid support for the stepwise incorporation of the N^a and side-protected amino acids Boc-Gly, Boc-N^G-Tos-L-Arg, Boc-L-(D)-Pro, Boc-trans-L-Hyp(OBzl), Boc-Cys(MeBzl), Boc-Asn, Boc-Phe, and Boc-Tyr. An individual cycle for each amino acid included deprotection of the Boc-group with 50% trifluoracetic acid in CH_2Cl_2, neutralization with 5% diisopropyl-ethylamine in CH_2Cl_2 and acylation with 5-fold excess of the protected amino acids. Between each operation, several extensive washings were performed with CH_2Cl_2-isopropyl alcohol and dimethylformamide. Boc-N^G-L-Tos-Arg, Boc-Hyp(O-Bzl) and Boc-Asn were coupled by the BtOH/DCC method[10]. Boc-Tyr was used unprotected and coupled with DCC in the same way as Boc-Gly (attachment to the resin), Boc-Cys(MeBzl), Boc-Phe and Boc-L-(D)-Pro. The completion of the acylation was determined by the ninhydrin test[11] and repeated couplings were undertaken if necessary. The fully protected peptides were cleaved from the resin with HF/anisole 9:1 for 1 h at 0 °C and the extracted crude peptides oxidized with $K_3Fe(CN)_6$.

The peptides were purified by partition chromatography and gel-filtration as previously described[9]. The overall yield was between 36–40% based on the initial 0.67 mequiv. amine groups/g resin. Analytical data for these analogs are shown in table 1.

Results and discussion. Antidiuretic and pressor activities of the 4- and 7-substituted proline and hydroxyproline analogs and some related compounds are shown in table 2. [4-L-Proline]AVP shows fairly high antidiuretic activity. [4-D-proline]AVP and [trans-4-hydroxyproline] AVP have little antidiuretic activity. The introduction of hydroxyproline into position 7, a substitution which when made alone increases antidiuretic activity to 712 U/mg[4], decreases the 90 U/mg activity of [4-proline]AVP to almost nothing. All four analogs have negligible pressor activities.

In a proposed model[17] for AVP receptor binding and activation, the amino acid side chains in position 3 and 4 (first β-turn) and 7 and 8 (second β-turn) are considered to be binding elements, free for intermolecular interactions with the receptor and having a limited effect in stabilizing backbone conformation. In this view these side chains can be more variable than the 'active' elements (Arg^8 in conjunction with GlyNH$_2$ and Asn^5) changes in which could destabilize the 3-dimensional integrity of the molecule.

Broad side chain alterations can be made in position 4 with retention of appreciable biological activity (see table 2). The carboxamide moiety of Gln seems not to be necessary. Amino acids with side chains of shorter than that of Gln, and with mostly lipophilic branched groups attached to the β-carbon, enhance activity. Such hydrophobic amino acids, although they do not 'force' β-turns[18,19], are thought to sta-