During the final stages of insulin biosynthesis in the pancreatic β-cell, C-peptide is enzymically cleaved from the precursor proinsulin molecule. The C-peptide and insulin are co-secreted by exocytosis (Rubenstein et al., 1969; Steiner et al., 1972; Beischer, 1983). In contrast to the established metabolic effects of insulin, the possible physiological effects of proinsulin C-peptide are unestablished. However, it has been suggested that this peptide contributes to the control of glucose homeostasis by reducing the release of insulin, glucagon and gastric inhibitory polypeptide, and by potentiating the hypoglycaemic action of insulin (Toyota et al., 1975, 1977; Wojcikowski et al., 1983). In contrast to the established class of specific binding sites exhibiting negative cooperativity (DeMeyts et al., 1983) or two or more classes of receptors with different but fixed affinities (Kahn, 1976).

Feedback inhibition of insulin release: a physiological role for proinsulin C-peptide?

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Table 1. Summary of reported effects of C-peptide on insulin secretion

<table>
<thead>
<tr>
<th>Species</th>
<th>C-peptide preparation (dose)</th>
<th>Effect of insulin release</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat (in vitro)</td>
<td>C-peptide I (100 ng/ml)</td>
<td>No effect</td>
<td>Toyota et al. (1975)</td>
</tr>
<tr>
<td>Rat (in vitro)</td>
<td>C-peptide I and II (100 ng/ml)</td>
<td>No effect</td>
<td>Toyota et al. (1975)</td>
</tr>
<tr>
<td>Rat (in vitro)</td>
<td>C-peptide I and II (300 ng/ml)</td>
<td>No effect</td>
<td>Wojcikowski et al. (1977)</td>
</tr>
<tr>
<td>Rat (in vitro)</td>
<td>C-peptide I and II (30 ng/ml)</td>
<td>No effect</td>
<td>Wojcikowski et al. (1977)</td>
</tr>
<tr>
<td>Rat (in vivo)</td>
<td>C-peptide I (10 ng-10 µg/min, i.v.)</td>
<td>No effect</td>
<td>Kaneko et al. (1979)</td>
</tr>
<tr>
<td>Rat (in vivo)</td>
<td>C-peptide I and II (500 µg/h per kg, i.v.)</td>
<td>No effect</td>
<td>Wojcikowski et al. (1983)</td>
</tr>
</tbody>
</table>

The isolated cells were cultured for 3 days at 37°C in a humidified atmosphere of 5% CO2. The culture medium was RPMI-1640 containing 11.1 mM glucose, 10% foetal calf serum with added antibiotics (100 units of penicillin/ml and 0.1 mg of streptomycin/ml). I125-C-peptide binding was measured in duplicate by incubating approximately 1.5 x 10^6 tumour β-cells (viability >90% assessed by Trypan Blue exclusion) in 100 µl of modified Krebs-Ringer-bicarbonate buffer (pH 7.4), containing 11.1 mM glucose and 5 mg of bovine serum albumin/ml (Flatt & Swanston-Flatt, 1985). The buffer was supplemented with synthetic tyrosylated rat C-peptide I (kindly donated by Professor N. Yanaihara, Shizuoka, Japan), iodinated by the method of Greenwood et al. (1963) to a specific activity of 2146 MBq/mg, and increasing concentrations (0–10 µg/ml) of unlabelled synthetic C-peptide I (N. Yanaihara). The cells were incubated for 30 min at 37°C in polyethylene microfuge tubes. At termination of incubation, the cells were separated by centrifugation through an underlying layer of oil into urea. Radioactivity in 20 µl aliquots of supernatant medium and in the cell pellet were counted in a gamma spectrometer. Specific binding was calculated by subtraction of non-specific binding (in the presence of 10 µg of unlabelled rat C-peptide/ml) from total binding.

Specific binding of iodinated rat C-peptide I to cultured β-cells during the 30 min incubations was 54 ± 6% (mean ± S.E.M. of eight determinations) of total binding. Preliminary experiments indicated that specific binding was maximum at this time and that degradation of the tracer over this period was <9%. Increasing concentrations of unlabelled rat C-peptide resulted in the progressive displacement of specifically bound iodinated rat C-peptide. Scatchard analysis of the binding data revealed a curvilinear plot with an upward concavity. This may reflect a single class of specific binding sites exhibiting negative co-operativity (DeMeyts et al., 1976) or two or more classes of receptors with different but fixed affinities (Kahn, 1976).

125I-C-peptide binding was measured in duplicate by incubating approximately 1.5 x 10^6 tumour β-cells (viability >90% assessed by Trypan Blue exclusion) in 100 µl of modified Krebs-Ringer-bicarbonate buffer (pH 7.4), containing 11.1 mM glucose and 5 mg of bovine serum albumin/ml (Flatt & Swanston-Flatt, 1985). The buffer was supplemented with synthetic tyrosylated rat C-peptide I (kindly donated by Professor N. Yanaihara, Shizuoka, Japan), iodinated by the method of Greenwood et al. (1963) to a specific activity of 2146 MBq/mg, and increasing concentrations (0–10 µg/ml) of unlabelled synthetic C-peptide I (N. Yanaihara). The cells were incubated for 30 min at 37°C in polyethylene microfuge tubes. At termination of incubation, the cells were separated by centrifugation through an underlying layer of oil into urea. Radioactivity in 20 µl aliquots of supernatant medium and in the cell pellet were counted in a gamma spectrometer. Specific binding was calculated by subtraction of non-specific binding (in the presence of 10 µg of unlabelled rat C-peptide/ml) from total binding.

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The apparent affinity constant, calculated as the concentration of rat C-peptide required to reduce specific binding of the tracer by 50%, was 200 pg/ml (66 pmol/l). The mean total number of binding sites was estimated at 5322 sites/cell. Based on a model of two classes of receptors, the high-affinity component represented approximately 8% of total specific binding.

In conclusion, several previous studies have suggested that proinsulin C-peptide affects glucose homoeostasis through actions including inhibition of glucose-stimulated insulin release (Table I). The present demonstration of specific binding sites for C-peptide on insulin-secreting cells from a transplantable rat insulinoma, is consistent with a possible physiological role for the connecting peptide of proinsulin.

Beischer, W. (1983) in 


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Radioimmunoassays (RIAs) for the antidiuretic hormone arginine vasopressin (AVP) in body fluids have often required tedious extraction procedures to remove non-specific substances that interfere with the immunoassay (Robertson et al., 1973; Fressinaud et al., 1974). Such procedures not only considerably increase the overall assay time, but may also, if not carefully monitored, lead to inaccuracies in results obtained. Here we describe details of a new sensitive RIA for the measurement of AVP in urine, that requires no prior extraction of the peptide.

The RIA was performed with an antibody (1:60000 final dilution) raised in rabbit against AVP (Ferring, Sweden) coupled to bovine thyroglobulin (Baylis & Heath, 1977). The AVP standard used was the 1st International Standard (NIBSC 77/501). A disjunct assay was performed, employing an overnight preincubation of antibody with unlabelled AVP, followed by 48 h with 125I-1AVP (5000 c.p.m.) at 4°C. Separation of bound from free was carried out by the second antibody procedure. Aliquots of 100 μl were used for each assay constituent (antibody, standard unknown and tracer).

Urine was obtained from adult human subjects in various states of hydration and immediately acidified with 20 μl of 5 M HCl per ml of urine. SepPak (C18) and florisil extractions of urine were carried out by methods previously described (Thomas & Lee, 1976; La Rochelle et al., 1980). Unextracted urine was first neutralized using microlitre amounts of 5 M NaOH before assay.

The cross-reactivity of the antibody (TG1) was 0.6%, < 0.1% and < 0.1% with arginine vasotocin, lysine vasopressin and oxytocin respectively. The sensitivity of the RIA was 1 pg/ml (0.1 pg/tube) in assay buffer [defined as the lowest standard that gives a binding level (B) 2.5 times the maximum binding, B0]. Table I gives the concentrations of urinary AVP obtained from 10 healthy subjects in various states of hydration. The difference between AVP concentrations in extracted and unextracted urine was found highly insignificant when subjected to a paired t-test analysis (P < 0.001). Unextracted and extracted urines always diluted in parallel to the standard curve.

Only two reports can be found on the RIA of AVP in unextracted urine (Oyama et al., 1971) or plasma (Fyhrquist et al., 1976). The results we report here are only a preliminary study, but do suggest that our assay system is also capable of measuring this hormone in unextracted samples.

This work was supported by a grant from the Wellcome Trust.


K. (1985)


Rubenstein, A. H., Clark, J. L., Melani, F. & Steiner, D. F. (1969)


Steiner, D. F., Kemmler, W., Clark, J. L., Oyer, P. E. & Rubenstein, A. H. (1972) in 


Wojcikowski, Cz., Fussganger, R. D. & Pfeiffer, E. F. (1977) in 

C-Peptide (Bayer, J., Krause, U. & Neagae, W., eds.), pp. 75–88, Schnetzer-Verlag, Konstanz.


Table I. AVP concentrations in extracted and unextracted urines

<table>
<thead>
<tr>
<th>Urine</th>
<th>Extracted</th>
<th>Unextracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>9.2</td>
</tr>
<tr>
<td>2</td>
<td>22.1</td>
<td>17.8</td>
</tr>
<tr>
<td>3</td>
<td>13.8</td>
<td>13.3</td>
</tr>
<tr>
<td>4</td>
<td>21.0</td>
<td>21.8</td>
</tr>
<tr>
<td>5</td>
<td>8.1</td>
<td>9.0</td>
</tr>
<tr>
<td>6</td>
<td>38.1</td>
<td>34.3</td>
</tr>
<tr>
<td>7</td>
<td>4.3</td>
<td>5.7</td>
</tr>
<tr>
<td>8</td>
<td>49</td>
<td>56.8</td>
</tr>
<tr>
<td>9*</td>
<td>15.5</td>
<td>17.0</td>
</tr>
<tr>
<td>10*</td>
<td>51.0 (51.32)</td>
<td>48.4</td>
</tr>
<tr>
<td>11†</td>
<td>9.6 (9.52)</td>
<td>9.0</td>
</tr>
</tbody>
</table>

*These subjects were especially dehydrated overnight, before urine collection.
†Extracted by the floral method.

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