Table 1 Active Synthesis of Ig Receptors for Antigen by B and T Lymphocytes

	Proportion of B cells in total population	ABC frequency $\times 10^{-5}$ * B	T	Groups compared	Statistical analysis P value † B	T
1 Untreated population	17%	69	28	1 : 2	<0.0005	<0.01
2 1 h after first capping with RIgG-α-MIgG	17%	0	0‡	2 : 3	<0.001	<0.005
3 18 h after first capping with RIgG-α-MIgG	9%	25	21	3 : 4	<0.001	<0.005
4 1 h after second capping with RIgG-α-MIgG	7%	0	0	1 : 3	<0.025 >0.40§	>0.60
5 1 h after RIgG-α-TVM	17%	84	47	1 : 5	>0.50	>0.30
6 18 h after RIgG-α-TVM	10%	23	21	1 : 6	<0.025 >0.50§	>0.50

* 50,000 to 75,000 lymphocytes were examined per group.
† Based on χ^2 test.
‡ One cell was found with ~thirty grains still in a small cap.
§ When corrections were made for the decrease in the overall proportion of B cells, which was found in two experiments out of five.

later resynthesised. Although there is no precedent for such co-capping, we sought formal proof that the antigen binding receptors which reappeared on B and T ABC were indeed immunoglobulin.

Two experiments were therefore extended by taking samples of cells known to have capped and resynthesised and exposing these cells once again to FITC-RIgG-α-MIgG. They were then, as before, put in capping conditions and later exposed, under non-capping conditions, to ^{125}I-TIGAL, followed by TRITC-RIgG-α-MIgG. Results of one complete experiment are given in Table 1: receptors for antigen on both B and T ABC which had been resynthesised after capping were cleared again by a second treatment with anti-Ig reagents. Samples of cells were examined before the total clearance of surface receptors by the second FITC-RIgG-α-MIgG treatment and showed that this clearance was also due to capping.

These experiments demonstrate the active synthesis of immunoglobulin receptors for antigen by T lymphocytes. The nature of these receptors in terms of known Ig classes remains to be investigated. It is perplexing that these receptors are not readily visualised using conventional fluorescent anti-Ig reagents. This may be due to their location in the T cell membrane; for instance, only a small part of the Ig receptor molecule(s) may be accessible to rabbit anti-M IgG antibody. These parts may, however, be detectable by antibody present in anti-M Ig antisera raised in chicken (F. L., J. R. L. Pink and L. Du Pasquier, manuscript in preparation).

We thank Drs B. A. Askonas, R. E. Cone, S. Fazekas de St Groth, and M. Nabholz for discussions. One of us (F. L.) is Chargé de Recherches du Fonds National Belge de la Recherche Scientifique (titre honoraire).

GEORGES E. ROELANTS
AINA RYDÉN
LENA-BRITT HÄGG
FRANCIS LOOR

Basel Institute for Immunology,
Grenzacherstrasse 487,
CH-4058 *Basel, Switzerland*

Received September 7, 1973.

[1] Roelants, G. E., Forni, L., and Pernis, B., *J. exp. Med.*, **137**, 1060 (1973).
[2] Taylor, R. B., Duffus, W. P. H., Raff, M. C., and de Petris, S., *Nature new Biol.*, **233**, 225 (1971).
[3] Loor, F., Forni, L., and Pernis, B., *Eur. J. Immun.*, **2**, 203 (1972).
[4] Preud'Homme, J. L., Neauport-Sautes, C., Piat, S., Silvestre, D., and Kourilsky, F. M., *Eur. J. Immun.*, **2**, 297 (1972).
[5] Neauport-Sautes, C., Lilly, F., Silvestre, D., and Kourilsky, F. M., *J. exp. Med.*, **137**, 511 (1973).
[6] Roelants, G. E., and Rydén, A., *Nature*, **247**, 104 (1974).
[7] Loor, F., and Kindred, B., *J. exp. Med.*, **138**, 1044 (1973).

Increase in Kallikrein Excretion during the Natriuresis produced by Arterial Infusion of Substance P

SUBSTANCE P was discovered by von Euler and Gaddum in 1931 in extracts of brain and small intestine[1] and its distribution and effects have recently been reviewed[2]. Its structure has been identified[3] and the undecapeptide has recently been synthesised[4]. Substance P is a potent stimulator of salivary secretion[5]; stimulation of the chorda-lingual nerve leads to increases in the production of saliva and blood flow through the salivary gland and is associated with the depletion of glandular kallikrein[6]. The presence of atropine blocks secretion but does not affect the increase in blood flow[7] or kallikrein depletion[6]. In homogenised nervous tissue substance P is located in the fraction containing the synaptic vesicles[8].

It seemed from these findings that substance P at the nerve endings might be released and lead to the activation or release of kallikrein. Kallikrein has been demonstrated in a variety of tissues which produce a liquid secretion such as salivary glands[9], small intestine[10], pancreas[11], sweat glands[12] and kidneys[13]. Urinary kallikrein resembles renal kallikrein[14] and differs from plasma kallikrein[15]. In severe renal failure urinary kallikrein excretion is very high which also supports the view that it is of renal origin and not filtered from plasma[16]. Kallikrein excretion is closely related to sodium excretion in a variety of circumstances[16–18]. Nevertheless increased sodium excretion also occurs without an associated increase in kallikrein excretion when there is a sharp fall in mineralocorticoid activity. These two processes related to sodium excretion are clearly differentiated by the Na/K ratio in the urine[19].

If 'substance P-inergic' nerves exist and promote the release of kallikrein, it might be expected that substance P infusion into the renal artery would lead to the release of kallikrein and a natriuresis. This has been tested in greyhound dogs under pentobarbital anaesthesia. Both ureters were catheterised and 0.9% saline was infused into the left renal artery at 1 ml min^{-1}. After several 10 min control collections of urine the infusion was changed to synthetic substance P (Beckman) in saline for three periods, then back to saline alone. Urinary sodium was measured by flame photometry. Kallikrein was assayed by determining the esterase activity of the enzyme in releasing tritiated methanol from a synthetic labelled substrate (*p*-tosyl-L-arginine-^{3}H-methyl ester)[20]. Pancreatic kallikrein (Bayer) was used as a standard in the assay and the results are given in esterase units, which are defined in Table 1.

In thirteen experiments on five dogs doses of 1, 10 and 100 ng substance P min^{-1} were used, equivalent to 30–4,300 pg kg^{-1} min^{-1}. The changes in sodium and kallikrein excretion, calculated as the differences between excretion in the experimental periods and the mean of the control periods immediately preceding and following the peptide infusion, are shown in the

Table 1 Increases in Sodium and Kallikrein Excretion in Response to Arterial Infusions of Substance P

Substance P dose (ng min^{-1})	Sodium excretion (μEq min^{-1})		P	Kallikrein excretion (mEU min^{-1})		P
	Control	Increase		Control	Increase	
1	38.8± 9.67 n (8)	11.7± 4.35 (12)	<0.025	411± 87 (8)	12±27 (12)	NS
10	43.8± 9.64 n (10)	67.3±14.73 (15)	<0.001	395± 98 (10)	127±33 (15)	<0.005
100	55.0±14.27 n (8)	128.9±19.16 (12)	<0.001	443±123 (8)	192±46 (12)	<0.005

Means ±s.e.
n, Number of control or experimental periods (see text).
P is derived from a *t* test on paired values.
EU, Esterase unit, equivalent to the hydrolysis of 4.8 μmol *p*-tosyl-L-arginine-methyl ester per h at *p*H 8.5 and 37° C.

table. The mean increases in sodium excretion were significant at each of the three doses. Mean kallikrein excretion increased at 1 ng min^{-1} though not significantly, but at 10 and 100 ng min^{-1} the increases were highly significant ($P<0.005$). The changes in excretion of sodium and kallikrein, when correlated with the logarithm of the dose of substance P infused, showed a highly significant correlation: $r=0.756$; $P<0.01$ for sodium and $r=0.735$; $P<0.01$ for kallikrein.

The data on sodium excretion place substance P amongst the most potent natriuretic substances so far described. Its threshold dose (around 1.0 ng min^{-1}) is less than, and its maximal effect greater than, those of the prostaglandins E_1, E_2 and A_1 found in comparable studies on dogs[21–23]. The concentration in the renal artery plasma which was natriuretic was approximately 0.1–1.0 ng ml^{-1} which is well below the limit of detection of a recent radioimmunoassay for substance P (ref. 24).

A variety of vasodilators are natriuretic when infused into the renal artery, for example bradykinin[25], lysyl-bradykinin[26], dopamine[27] and acetylcholine[28]. Substance P is also a vasodilator[29]. Since substance P stimulates natriuresis, salivary secretion and nasal secretion and all these processes are related to an increase in kallikrein activity, it is possible that the vasodilator action is dependent upon the release of kallikrein. There is strong evidence that urinary kallikrein originates from the kidney[14–16], being located principally in the cortex[30].

In view of the similarity between nervous stimulation and substance P infusion in their effects on stimulation of saliva flow and the related release of kallikrein and vasodilation in the salivary gland[5–7], the release of kallikrein from the kidney might be a direct effect of the substance P.

The effect of substance P on the kidney is a further example of the relation which has been shown between kallikrein excretion and sodium excretion[16–19] but in this case there is no associated expansion of body fluid volumes. The bradykinin released by kallikrein is known not only to be a vasodilator but also to increase vascular permeability. The natriuresis produced by the intra-renal release of kallikrein may be effected by vasodilator and permeability effects on the renal vasculature and tubules.

This work was carried out under a grant from the British Heart Foundation to I. H. M.

IVOR H. MILLS
N. A. A. MACFARLANE
P. E. WARD

Department of Investigative Medicine,
University of Cambridge,
Downing Street,
Cambridge CB2 1QN

Received June 13; revised November 15, 1973.

[1] von Euler, U. S., and Gaddum, J. H,. *J. Physiol. Lond.*, **72**, 74 (1931).
[2] Lembeck, F., and Zetler, G., *Int. Encycl. Pharmacol. Ther., Section 72*, **1**, 29 (Pergamon, New York, 1972).
[3] Chang, M. M., Leeman, S. E., and Niall, H. D., *Nature new Biol.*, **232**, 86 (1971).
[4] Tregear, G. W., Niall, H. D., Potts, J. T., jun., Leeman, S. E., and Chang, D. M., *Nature new Biol.*, **232**, 87 (1971).
[5] Vogler, K., Haefely, W., Hurlimann, A., Studer, R. O., Lergier, W., Strassle, R., and Berneis, K. H., *Ann. N.Y. Acad. Sci.*, **104**, 378 (1963).
[6] Gautvick, K. M., Nustad, K., and Vystyd, J., *Acta physiol. scand.*, **85**, 438 (1972).
[7] Heidenhain, R., *Pflüg. Arch. ges. Physiol.*, **5**, 309 (1872).
[8] Inouye, A., and Kataoka, K., *Nature*, **193**, 585 (1962).
[9] Werle, E., and von Roden, P., *Biochem Z.*, **286**, 213 (1936).
[10] Amundsen, E., and Nustad, K., *J. Physiol., Lond.*, **179**, 479 (1965).
[11] Kraut, H., Frey, E. K., and Werle, E., *Z. physiol. Chem.*, **189**, 97 (1930).
[12] Fox, R. H., and Hilton, S. M., *J. Physiol. Lond.*, **142**, 219 (1958).
[13] Carvalho, I. F., and Diniz, C. R., *Biochim. biophys. Acta*, **128**, 136 (1966).
[14] Nustad, K., *Br. J. Pharmac.*, **39**, 73 (1970).
[15] Webster, M. E., and Pierce, J. V., *Ann. N.Y. Acad. Sci.*, **104**, 91 (1963).
[16] Adetuyibi, A., and Mills, I. H., *Lancet*, ii, 203 (1972).
[17] Marin-Grez, M., and Carretero, O. A., *Physiologist*, **14**, 189 (1971).
[18] Marin-Grez, M., Cottone, P., and Carretero, O. A., *Am. J., Physiol.*, **223**, 794 (1972).
[19] Edwards, O. M., Adetuyibi, A., and Mills, I. H., *J. Endocrinol.*, **59**, xxxiv (1973).
[20] Beaven, V. H., Pierce, J. V., and Pisano, J. J., *Clin. chim. Acta*, **32**, 67 (1970).
[21] Johnston, H. H., Herzog, J. P., and Lauler, D. P., *Am. J. Physiol.*, **213**, 939 (1967).
[22] Vander, A. J., *Am. J. Physiol.*, **214**, 218 (1968).
[23] Herzog, J. P., Johnston, H. H., and Lauler, D. P., in *Prostaglandin Symp. Worc. Foundn. Exp. Biol.* (edit. by Ramwell and Shaw), 147 (Wiley, New York, 1968).
[24] Powell, D., Leeman, S., Tregear, G. W., Niall, H. D., and Potts, J. T., jun., *Nature new Biol.*, **241**, 252 (1973).
[25] Barraclough, M. A., and Mills, I. H., *Clin. Sci.*, **28**, 69 (1965).
[26] Webster, M. E., and Gilmore, J. P., *Am. J. Physiol.*, **206**, 714 (1964).
[27] Goldberg, L. I., McDonald, R. H., jun., and Zimmerman, A. M., *New Engl. J. Med.*, **269**, 1060 (1963).
[28] Vander, A. J., *Am. J. Physiol.*, **206**, 492 (1964).
[29] Pernow, B., *Ann. N.Y. Acad. Sci.*, **104**, 393 (1963).
[30] Nustad, K., *Br. J. Pharmac.*, **39**, 87 (1970).

Androgen-induced Sexual Differentiation of the Brain is Blocked by Inhibitors of DNA and RNA Synthesis

SEXUAL differentiation of gonadotrophin secretion in the rat is known to occur during the early postnatal period under the action of testicular androgens[1,2]. Animals of either sex if exposed to androgen during this critical period develop the