1 Georges, D., and Dubois, M.P., Archs Anat. microsc. Morph. exp. *68* (1979) 121.
2 Georges, D., and Dubois, M.P., C.r. Acad. Sci. Paris, Sér. D *290* (1980) 29.
3 Fritsch, H.A.R., Van Noorden, S., and Pearse, A.G.E., Cell Tissue Res. *202* (1979) 263.
4 Thorndyke, M.C., Regul. Peptides *3* (1982) 281.
5 Coons, A.H., Leduc, E.H., and Connolly, J.M., J. exp. Med. *102* (1955) 49.
6 Sternberger, L.A., Immunocytochemistry, John Wiley p.354. John Wiley, New York 1979.
7 Bargmann, W., Lindner, E., and Andres, K.H., Z. Zellforsch. *77* (1967) 282.
8 Snyder, S.H., Science *209* (1980) 976.
9 Mazzi, V., Boll. Zool. *19* (1952) 161.
10 Dawson, A.B., and Hisaw, F.L., J. Morph. *114* (1964) 411.
11 Thiebold, J., and Illoul, F., Bull. Soc. Hist. nat. Afr. N. *56* (1965) 87.
12 Chambost, M.D., Téthys *1* (1969) 901.
13 Lane, N.J., J. Ultrastruct. Res. *40* (1972) 480.
14 Bautz, A., Schilt, J., Richoux, J.P., and Dubois, M.P., C.r. Acad. Sci. Paris, Sér. D *291* (1980) 833.
15 Schilt, J., Richoux J.P., and Dubois, M.P., Gen. comp. Endocr. *43* (1981) 331.
16 Martin, G., and Dubois, M.P., Gen. comp. Endocr. *45* (1981) 125.

0014-4754/83/101156-03$1.50+0.20/0

Bombesin-like immunoreactivity in the pituitary gland

J. Major, M.A. Ghatei and S.R. Bloom

Department of Medicine, RPMS, Hammersmith Hospital, London, W12 OHS (England), March 22, 1983

Summary. Bombesin has been shown to stimulate release of anterior pituitary hormones both in vivo and in vitro. The aim of this study was to determine whether bombesin-like immunoreactivity could be detected in the human pituitary. Significant concentrations were found in the human anterior gland (4.6± 1.5 pmoles/g), posterior gland (1.5±0.4 pmoles/g) and stalk (8.1±0.8 pmoles/g). Significant amounts were also observed in the guinea-pig pituitary. Gel permeation chromatography revealed the presence of 2 major molecular forms of bombesin-like immunoreactivity, one co-eluting with porcine gastrin-releasing peptide and the other with amphibian bombesin.

A number of peptides which have been found in the brain and intestinal tract have been shown to be present in the pituitary and have stimulating effects on anterior pituitary hormone production. These include substance P[2-4], vasoactive intestinal polypeptide[5,6], somatostatin[3,7,8] and neurotensin[3,4,9,10].

Pharmacological studies have revealed that bombesin can also alter the release of anterior pituitary hormones, its effect apparently being dependent on the route of administration. For example, in the rat, i.v. injections of bombesin increases plasma levels of prolactin (PRL) and growth hormone (GH), whereas i.c.v. injection of the peptide inhibits stress induced PRL and basal GH secretion[3,10]. Bombesin also stimulates luteinising hormone (LH) release from incubated rat pituitary tissue[11], but as to whether PRL and GH release can be altered in a similar way is unclear[3,12]. A study using cultured bovine pituitary cells has shown that bombesin stimulates GH release, but fails to alter PRL secretion[13].

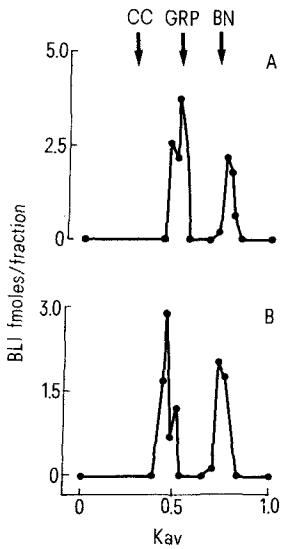
The aim of this study therefore, was to determine whether bombesin-like immunoreactivity (BLI) could be detected locally within the human and guinea-pig pituitary and to characterize this immunoreactivity chromatographically.

Materials and methods. Source of tissue. Human. Human pituitary tissue was collected post-mortem (mean age 80 years, range 34-95 years; mean autopsy delay 39 h, range 15-62 h) from patients who had died without neurological or psychiatric disease. The tissue was divided into the stalk, anterior and posterior lobes (n=6) and stored at −70 °C.

Animals. Adult male Duncan Hartley guinea-pigs (n=10) were killed by decaptitation and the pituitary glands removed.

Extraction. Each tissue was weighed and rapidly placed in boiling 0.5 M acetic acid (10 ml acid/g wet tissue) for 10 min[14]. They were then allowed to cool and stored at −20 °C until assay.

Radioimmunoassay. The tissue extracts were thawed and



Gel permeation chromatography profiles of guinea-pig (A) and human (B) pituitary extracts. CC, cytochrome C; GRP, gastrin-releasing peptide; BN, bombesin.

BLI in the human and guinea-pig pituitary (number of samples in parenthesis)

Species	Region	pmoles/g ± SEM
Human	Stalk (6)	8.1 ± 0.8
	Anterior (6)	4.6 ± 1.5
	Posterior (6)	1.5 ± 0.4
Guinea-pig	Whole (10)	18.09 ± 2.13

duplicate aliquots of 10 µl were assayed for BLI. The radioimmunoassay has been described in full elsewhere[15]. Briefly antiserum (BN 103) were raised in rabbits to [lys^3] - bombesin analogue conjugated to bovine serum albumin with glutaraldehyde and used at a final dilution of 1:640,000. This antibody crossreacted 92% with porcine bombesin-like, gastrin-releasing peptide (GRP), but showed no cross reactivity with other known gut and brain peptides. After 5 days incubation at 4 °C, free labeled peptide was separated from antibody bound peptide by charcoal precipitation. The assay was able to detect changes of 0.2 fmoles BLI/assay tube.

Chromatography. In order to study the molecular forms of BLI in the pituitary, extracts of tissue (0.5 ml) were applied separately to the top of a 0.9×60 cm Sephadex G-50 superfine column (Pharmacia). These were run at 3.5 ml/h at 4 °C and eluted with 0.5 M phosphate buffer, pH 7.4 containing 0.01 M EDTA, 0.2 M NaCl and 0.3% BSA. Prior to running the extracts the column was precalibrated with bombesin standard, dextran blue (mol.wt 2×10^6, Kav = 0), horse heart cytochrome C (mol.wt 12,384, Kav 0.25 ± 0.02) and $Na^{125}I$ (Kav = 1). The latter 3 were also run with each sample and acted as internal markers. The fractions between the void volume and 2 column volumes were assayed.

Treatment of data. BLI in the pituitary was expressed as the mean ± SEM. The elution coefficient (Kav) for each immunoreactive peak obtained by chromatography was calculated according to Laurent and Killander[16], and was used to compare the elution profiles from each column.

Results. The concentrations of BLI (pmoles/g wet wt) detected in the pituitary gland of the 2 species examined are shown in the table. In man, highest amounts were observed in the stalk (8.1 ± 0.8), moderate amounts in the anterior (4.6 ± 1.5) and lowest levels in the posterior pituitary (1.5 ± 0.4).

Gel permeation chromatography revealed the presence of 2 molecular forms of BLI, one co-eluting with the 27 amino acid peptide GRP and the other eluting just after the position of the smaller, 14 amino acid peptide amphibian bombesin (fig.). The elution positions for the 2 molecular forms were similar in both of the species examined, but their relative amounts differed. In the human pituitary approximately equimolar concentrations of both forms were observed, whereas, in the guinea-pig pituitary there was considerably more of the larger than the small form (representing 74% and 26% of total immunoreactivity respectivity). All recoveries were in excess of 70%.

Discussion. Our results show that BLI is found in the human and guinea-pig pituitary, the overall concentration being slightly higher in the freshly extracted guinea-pig tissue. However studies have shown that much of the neurochemical structure of the brain, including both neuropeptides and the classical neurotransmitters, remain stable during the average post mortem time to autopsy of 48 h[17,18], thus allowing a fair comparison to be made between the 2 species examined.

The concentration of BLI in the human pituitary was also found to be highest in the stalk and was significantly less in the posterior lobe compared to the anterior lobe. In the human and rat brain, the highest levels of BLI have been found in the hypothalamus[15,19]. It thus seems likely that mammalian anterior pituitary BLI is hypothalamic in origin and reaches the anterior lobe via the hypothalamic-hypophyseal portal system.

In the rat the regulation of pituitary hormone production by bombesin appears to be by more than 1 pathway, the peptide either acting directly on the gland, or via the CNS[3,10-12]. Studies in man, on the other hand, have so far produced uncertain results, since bombesin infusions have failed to alter LH, GH, TSH and FSH release, but PRL release may be stimulated[20,21]. The finding that BLI is present in the pituitary gland, and the possibility that bombesin can alter pituitary hormone production, justifies the need for further investigations into the peptide's physiological role.

1 Reprint requests to S.R. B., 2nd Floor Francis Fraser Labs, Hammersmith Hospital, Du Cane Road, London W12 OHS (England).
2 DePlatis, L.R., Fioruido, R.P., and Ho, R.H., Endocrinology *110* (1982) 282.
3 Rivier, C., Rivier, J., and Vale, W., Endocrinology *102* (1978) 519.
4 Vijayan, E., and McCann, S.M., Endocrinology *105* (1979) 64.
5 Morel, G., Besson, J., Rosselin, G., and Dubois, P.M., Neuroendocrinology *34* (1982) 85.
6 Vijayan, E., Samson, W.K., Said. S.I., and McCann, S.M., Endocrinology *104* (1979) 53.
7 Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J., and Guillemin, R., Science *179* (1973) 77.
8 Vale, W., Rivier, C., and Brown, M., A. Rev. Physiol. *39* (1977) 473.
9 Goedet, M., Lightman, S.L., Nagy, J.I., Marley, P.D., and Emson, P.G., Nature, Lond. *298* (1982) 163.
10 Tache, Y., Brown, M., and Collu, R., Endocrinology *105* (1979) 220.
11 Morley, J.E., Helmed, S., Briggs, J., Carlson, H.E., Hersham, J.M., Solomon, T.E., Lamers, C., and Damassa, D.A., Life Sci. *25* (1979) 1201.
12 Westendorf, J.M., and Schonbrunn. A., Endocrinology *110* (1982) 352.
13 Bicknell, R.J., and Chapman, C., Neuroendocrinology *36* (1983) 33.
14 Bryant, M.G., and Bloom, S.R., in: Radioimmunoassay of gut regulatory peptides, p.36. Eds S.R. Bloom and R.G. Long. Saunders, London 1982.
15 Ghatei, M.A., in: Radioimmunoassay of Gut Regulatory Peptides, p.131. Eds S.R. Bloom and R.G. Long. Saunders, London 1982.
16 Laurent, T.C., and Killander, J., J. Chromat. *14* (1964) 317.
17 Bird, E.D., in: Neurosecretion and brain peptides, p.657. Eds J.B. Martin, S. Reichlin and K.L. Bick. Raven Press, New York 1981.
18 Martin, J.B., and Landis, D.M.D., in: Neurosecretion and brain peptide, p.673. Eds J.B. Martin, S. Reichlin and K.L. Bick. Raven Press, New York 1981.
19 Ghatei, M.A., Uttenthal, L.O., Langevin, V., Rosser, M., and Bloom, S.R., 4th Int. Symp. Gast. Horms., Stockholm, Sweden, 1982, p.31.
20 Morley, J.E., Varner, A.A., Modlin, I.M., Carlson, H.E., Braunstein, G.D., Walsh, J.H., and Hershman, J.M., Clin. Endocr. *13* (1980) 369.
21 Pontiroli, A.E., Albertto, M., Restelli, L., and Facchinetti, A., J. clin. Endocr. Metab. *51* (1980) 1303.

0014-4754/83/101158-02$1.50 + 0.20/0