Our previous study has shown that GABA in the islets is mainly localized in the β-cells, because streptozotocin, a β-cell attacker in small doses, dramatically decreased the GABA level in the islets as well as causing the destruction of β-cells, and also because human insulinoma, a β-cell tumor, was found to contain a high amount of GABA[10]. Together with that report, our present result of low uptake of ^{3}H-GABA in the pancreatic islets indicates that GABA contained in the islets plays some functional role other than as a neurotransmitter as in the CNS.

1 Present address: Department of Anatomy, Institute of Basic Medical Sciences, University of Tsukuba, Niihari, Ibarakiken (Japan).
2 On leave from the Second Department of Internal Medicine, School of Medicine, Kobe University, Kobe (Japan).
3 M. Otsuka, in: The structure and function of nervous tissue, vol. 4, p. 249. Ed. G.H. Bourne. Academic Press, New York 1972.
4 Y. Okada, in: GABA in nervous system function, p. 235. Ed. E. Roberts, T.N. Chase and D.B. Tower. Raven Press, New York 1976.
5 N. Seiler and M. Wiechmann, Z. Physiol. Chem. *350,* 1493 (1969).
6 D.T. Whelan, C.R. Scriver and F. Mohyddin, Nature *224,* 916 (1969).
7 R.H. Drummond and A.T. Phillips, J. Neurochem. *23,* 1207 (1974).
8 G. Biel, E. Gylfe, B. Hellman and V. Neuhoff, Acta physiol. scand. *84,* 247 (1972).
9 Y. Okada, H. Taniguchi, Ch. Shimada and F. Kurosawa, Proc. Japan Acad. *51,* 760 (1975).
10 Y. Okada, H. Taniguchi and Ch. Shimada, Science *194,* 620 (1976).
11 T. Hökfelt and A. Ljungdahl, Exp. Brain Res. *14,* 354 (1972).
12 L.L. Iversen and J.S. Kelly, Biochem. Pharmac. *24,* 933 (1975).
13 P.E. Lacy and M. Kostianovsky, Diabetes *16,* 35 (1967).
14 Y. Okada and R. Hassler, Brain Res. *49,* 214 (1973).
15 T. Hökfelt, G. Johnston and A. Ljungdahl, Life Sci. *9,* 203 (1970).
16 T. Hattori, P.L. McGeer, H.C. Fibiger and E.G. McGeer, Brain Res. *54,* 103 (1973).
17 S.C. Woods and D. Porte, Jr, Physiol. Rev. *54,* 596 (1974).
18 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randle, J. biol. Chem. *193,* 265 (1951).

Increase in pituitary melanocyte-stimulating hormone activity of genetically obese (*ob/ob*) mice

Margaret H. Peaslee, Beatriz Moisset[1], H. Shibuya and Candace B. Pert

Department of Zoology, Louisiana Tech. University, Ruston (LA 71272, USA); Department of Psychology, Temple University, Philadelphia (PA 19122, USA); and Section of Biochemistry and Pharmacology, National Institutes of Mental Health, Bethesda (MD 20014, USA), 28 March 1979

Summary. Melanocyte-stimulating hormone (MSH) activity was measured in the pituitaries of genetically obese and lean control mice using the frog skin bioassay. Obese mice pituitaries demonstrated very significantly elevated levels of biologically active MSH when compared to their lean littermates. These results support the hypothesis that the elevated levels of pituitary hormones found in obese mice possess true biological activity.

The genetically obese mouse, C57BL/6J (*ob/ob*), presents a syndrome characterized by marked overeating, obesity, hyperinsulinemia, and mild hyperglycemia. The syndrome is caused by a single recessive gene *ob* located on chromosome 6[2]. It has been reported that obese mice demonstrate a 14-fold elevation of pituitary adrenocorticotropic hormone (ACTH) using radioimmunoassay[3], and there is similarly-obtained indirect evidence for increased levels of corticotropin-like intermediate lobe peptide (CLIP)[4]. Pituitary levels of β-endorphin, measured by radioimmunoassay, have recently been reported to be twice as high as that of lean controls[5]. Measurement of biologically active pituitary MSH levels in obese mice seemed appropriate.

The subjects were male obese C57BL/6J (*ob/ob*) mice of 3–5 months of age and their lean littermates (*ob/+* or *+/+*), received from the Jackson Laboratory, Bar Harbor, Maine, at weaning age (4 weeks) and maintained at a temperature of 23 °C on a 12-h light-dark cycle, with food and water ad libitum (Purina mouse chow). The mice were killed by rapid decapitation; their brains and pituitaries were dissected and frozen on dry ice. Individual pituitaries were homogenized in 2 ml of 2 N acetic acid with a Brinkmann polytron (setting 7.5, 15″) after 15 min heating in a boiling water bath and were centrifuged ag 12,000 × g for 20 min. The supernatant fluid was lyophilized to dryness and resuspended in 10 ml of 0.05 M phosphate buffer containing 0.25% bovine serum albumin and 0.5% mercaptoethanol, pH 7.5. Portions of the resuspend were used for the β-endorphin[5] and MSH assays.

Pituitary MSH levels were determined using the frog skin bioassay of Shizume et al.[6] with modifications by Peaslee and Milburn[7]. 16 frog skins, mounted on plastic rings and arranged by the Latin square grouping, were paled for 1 h in 4 20-ml rinses of frog Ringer's solution. 5 lambda aliquots of the pituitary resuspend were applied to each frog skin, using at least 2 skins for each pituitary. After 1 h the change in reflectance (skin darkening) produced by the pituitary resuspend was recorded with a Photovolt reflectance meter, model 670. The change in reflectance, measured in galvanometer units, was converted to units of MSH activity using a previously established standard curve[7]. Additional dilutions of the pituitary resuspend were prepared and bioassayed whenever exceptionally high readings made it necessary. A blank was prepared containing the phosphate buffer, bovine serum albumin, and mercaptoethanol and the effect of this solution on frog skin was noted. All data analyses comparing test and control

Pituitary MSH activity in genetically obese male mice

	Number of animals	Pituitary weight (mg)	Pituitary MSH activity $\times 10^{-2}$ (units/mg pituitary)
Obese (*ob/ob*)	11	1.6 ± 0.1	921 ± 147*
Lean controls (*ob/+* or *+/+*)	12	1.8 ± 0.2	173 ± 47

Numbers are means ± SEM. * $p < 0.001$.

mice responses were done using a t-test (Student's distribution) of mean differences.

As indicated in the table genetically obese mice (*ob/ob*) demonstrated a very significantly higher pituitary MSH activity than their lean littermates (*ob/+* or *+/+*). This is in agreement with Beevor et al. who demonstrated elevated α-MSH and CLIP levels in obese mice using radioimmunoassay. Since the frog skin bioassay could be described as measuring total biologically active MSH's as well as any melanophore-stimulating activity exhibited by ACTH it would be very interesting to know the pituitary MSH activity after neutralization with an anti-ACTH antibody which does not cross react with MSH. Using the results obtained by Edwardson and Hough[3] who measured pituitary ACTH of obese mice one can calculate the approximate portion of frog skin darkening produced by the intrinsic ACTH. Kastin et al.[8] propose that 1 milliunit of ACTH exerts an MSH activity of approximately 1 unit. Thus, the results demonstrated in this paper would be decreased by 2% for the lean controls and 0.6% for the obese mice if the frog skin darkening ability of ACTH were subtracted from the total.

The results lend further support to the concept that these pituitary polypeptides, namely ACTH, β-endorphin, CLIP, and the MSH's share a common precursor molecule[9,10]. It would appear that there is increased synthesis of this entire complex of pituitary polypeptides in the genetically obese mouse and that the MSH portion of this complex demonstrates full biological activity.

1 Present address: 341 Winding Way, Glenside, PA 19038, USA.
2 D.L. Coleman and K.P. Hummel, Diabetologia *9*, 287 (1973).
3 J.A. Edwardson and C.A.M. Hough, J. Endocr. *65*, 99 (1975).
4 S. Beevor, A. Beloff-Chain, A. Donaldson and J.A. Edwardson, J. Physiol. *275*, 55P (1977).
5 D.L. Margules, B. Moisset, M.J. Lewis, H. Shibuya and C.B. Pert, Science *202*, 988 (1978).
6 K. Shizume, A.B. Lerner and T.B. Fitzpatrick, Endocrinology *54*, 553 (1954).
7 M.H. Peaslee and S.E. Milburn, J. Endocr. *51*, 253 (1971).
8 A.J. Kastin, A. Arimura, S. Viosca, L. Barrett and A.V. Schally, Neuroendocrinology *2*, 200 (1967).
9 R.E. Mains, B.A. Eipper and N. Ling, Proc. natl Acad. Sci. USA *74*, 3014 (1977).
10 A.P. Scott, J.G. Ratcliffe, L.H. Rees, J. Landon, H.P.J. Bennet, P.J. Lowry and C. McMartin, Nature, New Biol. *244*, 65 (1973).

Effects of surgical sympathectomy on catecholamine concentrations in the posterior pituitary of the rat[1]

R.H. Alper, K.T. Demarest and K.E. Moore

Department of Pharmacology and Toxicology, Michigan State University, East Lansing (Michigan 48824, USA), 23 April 1979

Summary. Approximately one-third of the norepinephrine in the posterior pituitary of the rat is contained in terminals of sympathetic nerves which originate in the superior cervical ganglia; the remaining norepinephrine and dopamine appear to be in nerves of central origin.

Catecholamine-containing axon terminals have been demonstrated histochemically in the posterior pituitary of the rat and other mammalian species[2-6]. Dopamine (DA)-containing nerves terminating in this region appear to originate primarily in the arcuate nucleus of the hypothalamus; they constitute the tuberohypophyseal DA system[7]. There is controversy concerning the origin of norepinephrine (NE)-containing nerves in the posterior pituitary, but there have been suggestions that they are either of central origin[6] or that they represent sympathetic NE fibres originating in the superior cervical ganglia[5]. The differentiation of NE and DA nerves using histofluorescent techniques can be difficult, but recently developed radioenzymatic assays have made it possible to quantify the catecholamines in the posterior pituitary[8,9]. The present report describes the effects of bilateral superior cervical ganglionectomy (SCGX) on the NE and DA contents of several brain regions, including the posterior pituitary.

Methods. Male Sprague-Dawley rats (Spartan Research Animals, Inc., Haslett, MI) weighing 225-275 g were housed 3/cage in an animal room maintained at 24±1 °C, with automatic light from 07.00 to 21.00 h each day. Superior cervical ganglia were removed bilaterally under Equithesin anesthesia; in sham-control animals the ganglia were isolated, but not removed.

Animals were decapitated between 08.00 and 11.00 h 7 days after surgery. The median eminence was dissected from the hypothalamus as described previously[10]. The pituitary gland was removed from the sella turcica and, while being viewed under a dissecting microscope, the posterior pituitary (neurointermediate lobe) was teased away from the anterior pituitary. The median eminence, posterior pituitary and pineal gland were homogenized in 30 µl 0.2 N perchloric acid containing 10 mg% EGTA. The corpus striatum was dissected and homogenized in 50 vol. of the same 0.2 N perchloric acid solution. Homogenates were centrifuged in a Beckman Microfuge and DA and NE were analyzed in 10 µl aliquots of the supernatant using a modification of previously described radioenzymatic assays[11,12]. The protein content of the pellets was deter-

Effect of superior cervical ganglionectomy on catecholamine concentrations in various brain regions

Treatment	No. of Animals	Dopamine (ng/mg protein) Median eminence	Posterior pituitary	Striatum	Norepinephrine (ng/mg protein) Median eminence	Posterior pituitary	Pineal
Sham-controls	15	112.3±7.4	6.3±0.3	100.9±3.5	52.7±4.4	2.7±0.3	3.8±0.4
Ganglionectomized	10	111.9±9.0	6.8±0.3	94.8±3.8	41.4±5.1	1.7±0.3*	<0.5**

Values represent means ±1 SE. Animals were operated 7 days prior to sacrifice. *Significantly different ($p<0.01$) from sham-controls. **The minimal concentration of NE that could be measured in a single pineal gland.