the validity of the hypothesis that plant hormones serve as biochemical signals to regulate insect reproduction seasonally.

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Uptake of ^{3}H-GABA (γ-aminobutyric acid) and ^{3}H-leucine in the pancreatic islets and substantia nigra of the rat

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Summary. Isolated pancreatic islets and thin slices of substantia nigra (SN) of the rat were incubated in a medium containing ^{3}H-GABA or ^{3}H-leucine to test the activity of both tissues in the uptake of those substances. Pancreatic islets showed a low uptake of both ^{3}H-GABA and ^{3}H-leucine, but SN had a high activity in the uptake of ^{3}H-GABA, though not for ^{3}H-leucine. This suggests that GABA contained at high levels in the pancreatic islets plays some functional role other than in neurotransmission as in the central nervous system (CNS).

γ-Aminobutyric acid (GABA) and its synthesizing enzyme, glutamate decarboxylase (GAD), have been mainly found in the invertebrate and vertebrate nervous system, where GABA functions as an inhibitory neurotransmitter[3, 4]. On the other hand, GABA and GAD have also been found in non-neuronal tissues such as kidney, liver, blood vessel, pancreas and pancreatic islets, although at much lower concentrations than in nerve tissue[5–8]. In the previous study from our laboratory, as high a concentration of GABA and as high a GAD activity as in the central nervous system (CNS) were found in rat pancreatic islets and in human insulinoma[9, 10]. In brain tissue a high level of uptake of ^{3}H-GABA into glial cells and nerve terminals has been reported, and this uptake is considered to be important for the inactivation and reutilization of GABA as the neurotransmitter[11, 12]. In this respect it seems worthwhile to investigate whether or not GABA in the pancreatic islets functions in the same manner as in the nervous system. In this paper the uptake of ^{3}H-GABA and ^{3}H-leucine in isolated pancreatic islets was studied in comparison with that of a thin slice of substantia nigra (SN) which contains the highest amount of GABA in the CNS[4].

Materials and methods. Albino Wistar rats (200–300 g) were anesthetized with pentobarbiton sodium (50 mg/ml/kg) and the abdominal cavity was opened. The pancreas was carefully excised after the injection of 10 ml of Krebs Ringer solution containing collagenase (25 mg/10 ml, Boehringer Mannheim) into the pancreas through the choledox duct. The pancreas was chopped and incubated at 37 °C with mechanical stirring to separate the islets from exocrine gland. This isolation of the islets was performed with a minor modification, according to the method of Lacy and Kostianovsky[13]. The skull was opened and the brain was removed under the same anesthesia. Using a glass guide and a razor blade a thin section (400 µm in thickness) of SN was prepared[14] and the weight was determined with a torsion balance. After preincubation of the isolated islets and the SN slice for 20 min in 0.5 ml of standard medium (concentration in mM: NaCl 125, KCl 5, KH_2PO_4 1.24, $MgSO_3$ 1.3, $NaHCO_3$ 26, $CaCl_2$ 1.3, glucose for pancreatic islets 3.3 and for SN slice 8.0), stirred and kept under a 95% O_2 and CO_2 atmosphere, the tissues were further incubated for 50 min in the standard medium containing radioactive substances for the uptake study. For the autoradiographic study, the pancreas tissue was chopped into pieces (0.5–1 mm^2) using a razor blade. The chopped pancreas tissue and the SN slice were incubated together in the same medium in the manner described above. The temperature of the medium was kept at 37 °C throughout the experiment.

Results and discussion. Thin slices of SN and isolated pancreatic islets were incubated in the medium containing ^{3}H-GABA or ^{3}H-leucine. As indicated in the table, the SN slice showed a high activity in the uptake of ^{3}H-GABA with a high uptake ratio, whereas the pancreatic islets showed very low uptake. The high uptake of ^{3}H-GABA in SN confirmed the results of previous studies[14–16]. In contrast to the high uptake of ^{3}H-GABA by SN, both SN and pancreatic islets showed a low uptake of ^{3}H-leucine, with similar ratios of uptake, as indicated in the table.

In the autoradiograms of SN, the light microscopic study revealed that silver grains were found abundantly in the SN except in the regions of neuron soma, which were not densely labelled (figure, A). On the other hand, in the chopped pancreas, there was considerably less labelling in both exocrine and endocrine glands (figure, B). Exocrine acini were slightly more highly labelled than the islets. Silver grains counted 15.0 ± 3.7 (SD)/10 μm^2 ($n = 120$) in acini and $7 \pm 3.9/10$ μm^2 ($n = 80$) in the endocrine islets. Thus, the autoradiographic study confirmed the result of the biochemical uptake study mentioned above. It is interesting, however, to note that some heavily labelled cells were observed within the pancreatic islets (arrows in the figure, B). The labelled cells were mostly located in the outer regions of the islets. The determination of the type of these cells is now under investigation by electron microscopic examination, which is revealing that these cells may belong to the D-type cells in the pancreatic islets. Those cells which showed high uptake of ^{3}H-GABA may be involved in the function of the pancreatic islets.

The high amount of GABA contained in the SN is considered to be localized in the GABAergic nerve endings densely terminating in the SN and to function as an inhibitory neurotransmitter[4]. In CNS a high affinity for the uptake of GABA in nerve terminals and glial cells is apparently needed for the inactivation of the GABA effect, and its re-utilization[12]. There have been many reports of studies of the pancreatic islets showing the existence of sympathetic and parasympathetic innervation to the islets which more or less influences the release of insulin[17]. However, no GABAergic innervation has been reported.

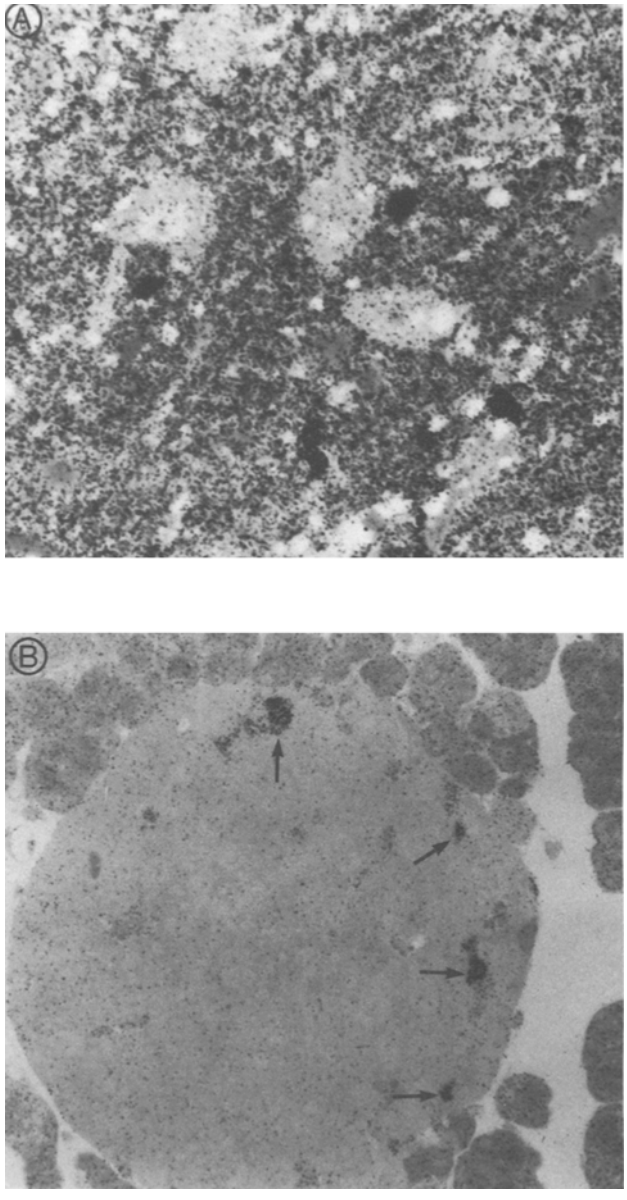
Uptake of ^{3}H-GABA and ^{3}H-leucine in the isolated pancreatic islets and the slice of substantia nigra of the rat

	^{3}H-GABA	^{3}H-leucine
Substantia nigra	32.13 ± 2.84 ($n = 4$)	3.98 ± 0.16 ($n = 3$)
Isolated islets	1.67 ± 0.21 ($n = 5$)	3.93 ± 1.10 ($n = 3$)

A thin SN slice (about 1 mg) and pancreatic islets (40 islets were used for each experiment) were incubated in 0.5 ml medium containing^{3}H-GABA ([2-3-^{3}H]-GABA, New England Nuclear, S. act. 39.2 Ci/mmole) with the final concentration at 5×10^{-7} M in the presence of aminooxyacetic acid (AOAA) at a concentration of 10^{-5} M, or in medium containing ^{3}H-leucine (L-[4-5-^{3}H]-leucine, New England Nuclear, S. act. 58 Ci/mmole) with final concentration at 5×10^{-7} M. 5 µl of the labelled medium was taken into a scintillation vial, and 10 ml of dioxan scintillation solution was added, and the radioactivity in the medium was determined using a Liquid Scintillation Spectrometer. After the incubation of the tissues, they were washed 3 times with isotope-free medium. 0.5 ml of soluent-350 (Packard) was added to the SN slice and it was incubated at 38 °C for 20 min to dissolve the tissue. 10 ml of toluene scintillation solution was added to it and the radioactivity was determined. In the case of pancreatic islets, after incubation with the labelled substance and washing with isotopefree medium, they were homogenized with 200 µl of water and half of the homogenate was used for the determination of radioactivity and the rest for the protein assay. For the determination of radioactivity, the homogenate was dried and 0.5 ml of soluent-350 was added and incubated for 20 min at 38 °C to dissolve the tissue. After the addition of 10 ml of toluene scintillation solution, the radioactivity was read by scintillation counter. The content of protein was determined by the method of Lowry et al.[18]. The protein content of the homogenate for which the radioactivity was determined was calculated from the protein content of the other half of the homogenate. The values in the table indicate the uptake ratio which is estimated as follows:

$$\text{uptake ratio} = \frac{\text{cpm of the incubated tissue/mg wet weight (for SN) or 0.1 mg protein (for islets)}}{\text{cpm of the incubation medium/µl}}$$

n, number of experiments.



Light microscopic autoradiograms from the slice of substantia nigra (A) and the chopped pancreas (B) after incubation with ^{3}H-GABA. The SN slice and chopped pancreas were incubated for 50 min in the standard medium containing ^{3}H-GABA or ^{3}H-leucine at a final concentration of 1×10^{-6} M. After the incubation they were washed with radioisotope-free medium and fixed with 5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) overnight. Tissues were postfixed with 2% osmium tetroxide in the same buffer for 2 h and dehydrated with graded ethanol and embedded in Epon. Semithin sections (1 µm thick) were cut on a MT-2 ultratome, and were placed on gelatin-coated glass slides, then coated by flooding or dipping methods with Sakura M_2 emulsion diluted 1:1. Following exposure for 1 month at 4 °C, the autoradiograms were developed in Konidol-X for 4 min at 20 °C and fixed with Super Fuji Fix. Some of the autoradiograms were stained with 0.1% toluidine blue in 0.1 M phosphate buffer. In (A) densely distributed silver grains are observed in the SN, although nigral cell bodies were not labelled. In the pancreas (B) both exocrine and endocrine glands are not densely labelled as seen in SN. It is to be noted however, that there are some heavily labelled cells in the outer part of the pancreatic islet (arrows). Horizontal bar at right bottom indicates 50 µm both A and B.

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.

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

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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. * $p < 0.001$.