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Effects of Alterations in the Activity of Tuberohypophysial Dopaminergic Neurons on the Secretion of α-Melanocyte Stimulating Hormone (42735)

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Abstract. Administration of γ-butyrolactone (GBL), an anesthetic which reduces dopaminergic neuronal activity, decreased the concentration of the dopamine (DA) metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in the intermediate lobe of the pituitary gland, and increased α-melanocyte stimulating hormone (αMSH) concentrations in the serum of male rats. Bilateral electrical stimulation of the rostral arcuate nucleus, which contains perikarya of tuberohypophysial DA neurons, increased DOPAC concentrations in the intermediate lobe and decreased αMSH concentrations in the serum of GBL-anesthetized rats. Administration of the DA antagonist haloperidol prevented the decline in serum αMSH levels following arcuate nucleus stimulation, but had no effect on serum αMSH concentrations in sham-stimulated GBL-treated rats. These results indicate that GBL-induced decreases or stimulation-induced increases in the activity of tuberohypophysial DA neurons are accompanied by corresponding changes in the metabolism of DA in the intermediate lobe of the rat pituitary gland, and by reciprocal changes in the secretion of αMSH.

Tuberohypophysial dopamine (DA) neurons located in the rostral arcuate nucleus have axons that project through the median eminence and terminate in the intermediate and neural lobes of the rat pituitary gland (1). In the intermediate lobe, these neurons make synaptic contact with pro-opiomelanocortin (POMC) peptide-synthesizing melanotrophs (2–4). The secretion of POMC-derived peptides, including α-melanocyte stimulating hormone (αMSH) is regulated, at least in part, by tonic dopaminergic inhibitory tone (4–7). Much of what is known about the inhibitory effects of DA on αMSH secretion is based upon studies performed *in vitro*. DA, either applied directly to dispersed intermediate lobe cells or added to solutions perfusing intact neurointermediate lobes, inhibits the release of αMSH (3, 5, 8). Furthermore, electrical stimulation of the mediobasal hypothalamus in hypothalamo-hypophysial explants containing tuberohypophysial DA neurons decreases the secretion of αMSH (9). On the other hand, the DA receptor antagonist sulpiride blocks the decrease in basal αMSH secretion following *in vitro* electrical stimulation of the pituitary stalk (9). The administration of DA antagonists increase and DA agonists decrease serum αMSH concentrations (4–7), but little information is available regarding the role of tuberohypophysial DA neurons in regulating the secretion of αMSH *in vivo*.

In the present study the effects of altering impulse flow in tuberohypophysial DA neurons were examined on 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations in the intermediate lobe of the rat pituitary gland and on αMSH concentrations in the serum. The results reveal that procedures that decrease or increase the activity of tuberohypophysial DA neurons *in vivo* produce corresponding changes in intermediate lobe DOPAC concentrations and reciprocal changes in serum αMSH concentrations. These data suggest that DA released from tuberohypophysial neurons *in vivo* tonically inhibits the secretion of αMSH from the intermediate lobe.

Materials and Methods. *Animals.* Male Long–Evans rats (200–225 g) were obtained from Charles River Laboratories (Wilmington, MA) and maintained under conditions of controlled temperature and lighting (illumination 0700 to 1900 hr). Rats were housed four per cage and supplied with food and water *ad libitum*.

Electrical stimulation of the arcuate nucleus. Rats were anesthetized with γ-butyrolactone (GBL, 1000 mg/kg, ip, Sigma Chem-

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ical Co., St. Louis, MO) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). Coaxial stainless-steel electrodes (diameter 0.25 mm, SNE-100, Rhodes Medical, Woodland Hills, CA) with the cathodal center and anodal shaft contacts exposed 0.25 mm were placed bilaterally in the arcuate nucleus. Using the atlas of König and Klippel (10) as a reference, the coordinates of the electrode tips were approximately A 5.0 mm, L ±0.3 mm, V −3.6 mm. The arcuate nucleus was stimulated for 15 min with twin biphasic pulses of 1 msec duration, 200 μA peak-to-peak intensity, at a frequency of 10 Hz unless otherwise noted. The current was generated by two Grass stimulators (S-9 and SD-9, Grass Instruments, Quincy, MA) and continuously monitored through an oscilloscope. At these stimulation parameters, there was no histological evidence of electrolytic damage to the tissue.

Tissue dissection. Following the appropriate treatments, animals were decapitated and trunk blood was collected in test tubes on ice. Trunk blood was allowed to clot, and centrifuged, and serum was collected and stored −20°C until assayed. Brains and pituitary glands were removed from the skull and frozen on aluminum foil placed directly over dry ice. Intermediate lobes were dissected from whole pituitary glands as described previously (11), and placed in 60 μl of 0.1 *M* phosphate-citrate buffer (pH 2.5) containing 15% methanol and stored at −20°C. Frontal brain sections (600 μm) were prepared in a cryostat and electrode placement was determined in thaw-mounted brain sections under a microscope. Data from animals in which the electrodes were not in close proximity to the arcuate nuclei were discarded.

Assays. DOPAC concentrations in intermediate lobe samples were determined by high-performance liquid chromatography with electrochemical detection as described previously (12). Tissue pellets were dissolved in 1.0 *N* NaOH and assayed for protein content (13).

The concentrations of αMSH were measured in serum by a double antibody radioimmunoassay. Antisera to αMSH were kindly supplied by Dr. G. Mueller, Uniformed Services University for the Health Sciences (Bethesda, MD). These antisera cross-react on an equimolar basis with desacetyl-αMSH and diacetyl-αMSH; they do not detect up to 30 ng/tube of any of the following: deaminated αMSH, βMSH, ACTH 1–10, 1–13, or 1–24, β-END peptides, or β-LPH. The assay procedure is a modification of that described by Pettibone and Mueller (14). Using an aliquot of 250 μl, the assay sensitivity was 35 pg/ml and the intraassay and interassay variations were approximately 9 and 13%, respectively.

Statistics. Statistical analyses were conducted using Student's *t* test for comparisons between two groups, and analysis of variance followed by Student–Newman–Keuls test for comparisons among three or more groups (15). Differences were considered significant if the probability of error was less than 5%.

Results. As shown in Fig. 1, 35 min following administration of an anesthetic dose of GBL (1000 mg/kg) there was a decrease in DOPAC concentrations in the intermediate lobe and a concomitant increase in αMSH concentrations in the serum. These results suggest that GBL decreases impulse flow in tuberohypophysial DA neurons, and are consistent with a general inhibitory action of GBL on DA neurons in other regions of the brain (16–18). Furthermore, the GBL-in-

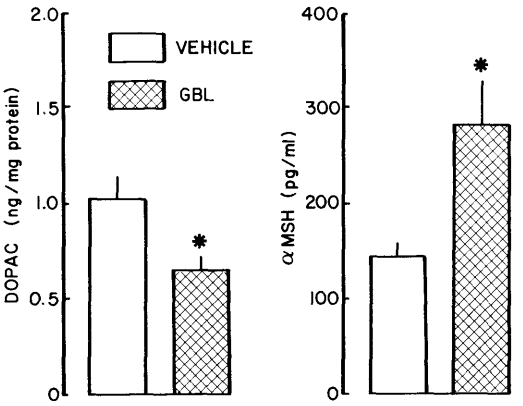

FIG. 1. The effects of administration of GBL on DOPAC concentrations in the intermediate lobe of the pituitary and on serum concentrations of αMSH. Animals were injected ip with either vehicle (0.9% saline; 1 ml/kg) or GBL (1000 mg/kg) 35 min before sacrifice. Each column represents the mean and the vertical line 1 SE of seven to eight animals. *Values that are significantly different from those of vehicle-injected animals ($P < 0.05$).

duced increase in circulating levels of αMSH is consistent with a tonic inhibitory effect tuberohypophysial DA neurons on the secretion of this hormone (4, 8).

Bilateral stimulation of the arcuate nucleus increases the synthesis and turnover of DA in the intermediate lobe of the pituitary gland in GBL-anesthetized rats (19), and this effect is presumably due to electrical activation of tuberohypophysial DA neuronal cell bodies located in the arcuate nucleus. To determine the appropriate stimulation intensity for maximal activation of tuberohypophysial DA neurons, the arcuate nucleus was stimulated in GBL-treated rats at 100, 200, and 400 μA for 15 min, and intermediate lobe DOPAC concentrations and serum αMSH concentrations were determined. The results from these preliminary experiments demonstrated that the maximal effect on these parameters occurs with 200 μA of current (DOPAC concentrations were 134 ± 10% and serum αMSH concentrations were 54 ± 5% of sham-stimulated controls, n = 8 per group). In all subsequent experiments this stimulus intensity was employed.

The time course of the effects of electrical stimulation of the arcuate nucleus is depicted in Fig. 2. DOPAC concentrations were significantly increased in the intermediate lobe after 10 and 15 min of stimulation, and this was accompanied by a concurrent decrease in serum αMSH concentrations. To determine if the decline in serum αMSH concentrations following arcuate nucleus stimulation is due to increased DA release from tuberohypophysial neurons, the DA antagonist haloperidol (1.0 mg/kg; ip) was administered 30 min prior to the onset of arcuate nucleus stimulation in GBL-anesthetized rats. Consistent with the results presented in Fig. 2, stimulation of the arcuate nucleus for 15 min decreased serum αMSH concentrations in GBL-treated rats, and this effect was blocked by haloperidol (Fig. 3). These results suggest that the decrease in αMSH secretion following stimulation of the arcuate nucleus is due to an increase in DA release from intermediate lobe tuberohypophysial DA neurons.

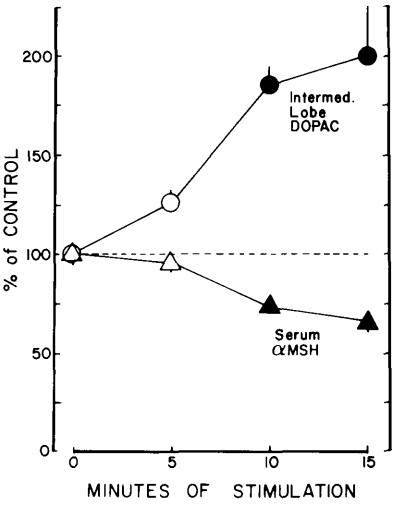

FIG. 2. Time course of the effects of arcuate nucleus stimulation on DOPAC concentrations in the intermediate lobe of the pituitary and serum αMSH levels. Animals were anesthetized with GBL (1000 mg/kg; ip) 20 min prior to stimulus onset and received electrical stimulation (200 μA intensity) for 5, 10, or 15 min. Control animals received sham stimulation for 15 min. Each symbol represents the mean and the vertical line 1 SE of 7–10 animals. Values are expressed as a percentage of values in sham-stimulated animals; absolute values in sham animals were 0.73 ± 0.05 ng/mg protein for DOPAC concentrations in the intermediate lobe, and 265 ± 18 pg/ml for serum αMSH. Solid symbols represent values that are significantly different from those of sham-stimulated animals ($P < 0.05$).

Discussion. The rate of DA metabolism in the striatum is coupled to the activity of nigrostriatal DA neurons, and pharmacological and electrophysiological procedures that activate or inhibit these neurons produce corresponding changes in DOPAC concentrations in this brain region (17, 18). Similarly, procedures that increase or decrease the activity of tuberoinfundibular dopaminergic neurons produce corresponding changes in DOPAC concentrations in the median eminence (16, 20). The results from the present study reveal that alterations in impulse flow in tuberohypophysial DA neurons produce corresponding changes in DA metabolism in the intermediate lobe. These results are in agreement with the *in vitro* experiments of Racke and co-workers (21) who demonstrated that the release of DOPAC from the neurointermediate lobe is increased following electrical stimulation of the pituitary stalk. Taken together these results suggest that DOPAC concentrations in

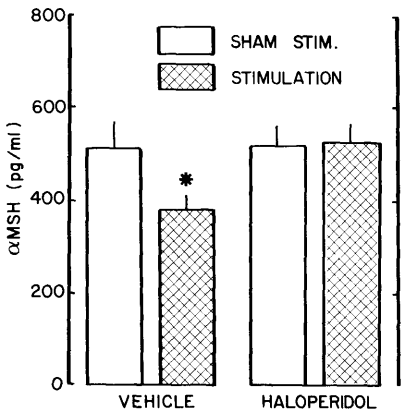

FIG. 3. The effects of haloperidol on the stimulation-induced decreases in serum concentrations of αMSH. Animals were injected sc with either vehicle (0.3% tartaric acid; 1 ml/kg) or haloperidol (1 mg/kg) 30 min prior to stimulus onset and anesthetized with GBL (1000 mg/kg; ip) 20 min prior to stimulus onset. Animals received either sham or arcuate nucleus stimulation (200 μA intensity) for 15 min. Each column represents the mean and the vertical line 1 SE of 5–10 animals. *Value that is significantly different from those of sham-stimulated animals ($P < 0.05$).

the intermediate lobe reflect the activity of tuberohypophysial DA neurons.

The ability of GBL to increase serum αMSH concentrations in the present study is consistent with a tonic inhibitory role of tuberohypophysial DA neurons on αMSH secretion (4, 8). GBL inhibits the activity of DA neurons in the brain (17, 19, 22, 23) and reduces the rate of DA synthesis and turnover in tuberohypophysial DA neurons (19, 21). Presumably by inhibiting DA release from these neurons, GBL decreases the amount of DA available to interact with DA receptors on intermediate lobe melanocytes and this results in an increase in αMSH secretion. The inability of haloperidol to further increase serum αMSH concentrations in GBL-treated rats (Fig. 3) suggests that the tonic inhibitory effects of DA have been removed following the administration of GBL.

Electrical stimulation of the arcuate nucleus reduces serum αMSH concentrations in GBL-treated rats, and these results are consistent with the *in vitro* work of Davis (9) who demonstrated that electrical stimulation of the mediobasal hypothalamus inhibits αMSH secretion from hypothalamo-hypophysial explants. In both the present study and in that by Davis, administration of a DA antagonist blocked the stimulation-induced decrease in αMSH secretion. Taken together, these data indicate that the inhibitory effect of arcuate nucleus stimulation on αMSH secretion is due to an increase in DA release from tuberohypophysial DA neurons.

In addition to inhibiting αMSH secretion, DA inhibits the secretion of other POMC peptides from the intermediate lobe (3, 24, 25). Chronic treatment with the DA agonist bromocriptine decreases the content of POMC mRNA and the activity of peptide acetyltransferase, an enzyme involved in processing of POMC peptides, in the intermediate lobe (26). Conversely, chronic treatment with the DA antagonist haloperidol has the opposite effects on POMC mRNA and peptide acetyltransferase activity (26). Accordingly, tuberohypophysial DA neurons may play a role in the regulation of the synthesis, processing, and secretion of POMC peptides from intermediate lobe melanocytes.

In summary, the results of the present *in vivo* study indicate that alterations in impulse flow in tuberohypophysial DA neurons are accompanied by corresponding changes in the metabolism of DA in the intermediate lobe and reciprocal changes in αMSH concentrations in the serum.

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