Biogenic Amines and Control of Melanophore Stimulating Hormone Release

Abstract. *Release of melanophore stimulating hormone (MSH) from the vertebrate pars intermedia is under inhibitory control by the hypothalamus. Removal of the rat pituitary or the neurointermediate lobe of the frog* (Rana pipiens) *to in vitro incubation medium results in rapid uninhibited release of MSH. This secretion is inhibited by norepinephrine, epinephrine, phenylephrine, and dopamine, and the inhibition is antagonized by α-adrenergic receptor blocking agents. Isoproterenol stimulation of MSH secretion from isolated glands is blocked by propranolol, a β-adrenergic receptor antagonist. These results implicate dopaminergic or classical α-adrenergic receptors (or both) in inhibition of MSH release by catecholamines, and implicate β-adrenergic receptors in stimulation of MSH release by the bioamines.*

Biogenic amines are implicated (*1*) in the regulation of pituitary hormone secretion. Their effects, however, are considered to be indirect in that they apparently control the release of hypophysiotropic factors, which then directly stimulate or inhibit pituitary secretion. However, catecholamines directly affect adenohypophysial hormone release in vitro. It has been reported that release of follicle stimulating hormone (*2*) and thyrotropin (*3*) is stimulated by catecholamines whereas release of prolactin (*4*, *5*) and melanophore stimulating hormone (MSH) (*6*) is inhibited. Other studies on release of follicle stimulating hormone (*7*), prolactin (*8*, *9*), and MSH (*10*) have provided conflicting data. It has been suggested that the effects of catecholamines on pituitary release of prolactin and other hormones are nonspecific (*9*).

We report here that catecholamines can both stimulate and inhibit MSH release from the vertebrate pituitary in vitro and do so through classical adrenergic receptor mechanisms.

Isolated pituitaries of the rat (Sprague-Dawley) and neurointermediate lobes of the frog (*Rana pipiens*) were studied (*11*). The release of MSH from a control set of pituitaries into incubation medium was compared to that from a similar set of pituitaries incubated with a hormone or other agent. The MSH released into the incubation medium during a 15-, 30-, or 60-minute incubation was bioassayed on frog skins by a photoreflectance method (*12*). Statistical differences in hormone release between experimental means were determined by Student's *t*-test.

Removal of the rat pituitary or frog neurointermediate lobe from its connection with the hypothalamus and subsequent transfer to an incubation medium resulted in rapid release of MSH. This release was completely inhibited in the presence of epinephrine (E), norepinephrine (NE), and dopamine (DA), and could be demonstrated within 15 minutes of incubation (Fig. 1). At lower concentrations (10^{-6} to $10^{-7}M$), E stimulated release of MSH (Fig. 1A). Phenylephrine at a high concentration ($10^{-4}M$) also inhibited MSH secretion from both frog and rat pituitary in vitro. Isoproterenol, on the other hand,

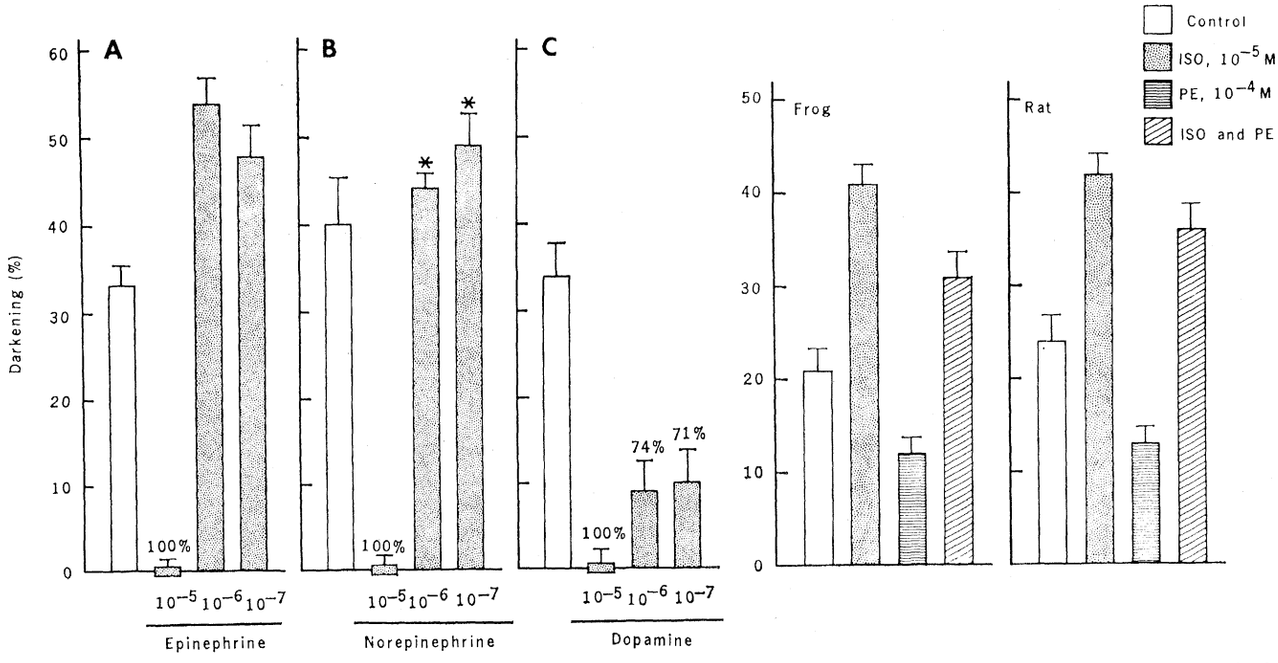

Fig. 1 (left). Bioassay of MSH released from frog neurointermediate lobes in vitro during incubation with catecholamines (stippled bars) compared to that released from a control set of pituitaries (open bar) in each experiment. Each value represents the darkening response (mean ± standard error) of eight frog skins to the MSH released from four glands under each experimental condition. Incubations lasted 1 hour in (A) and (B) and 15 minutes in (C). For each catecholamine, inhibition of MSH release could be demonstrated after either 15, 30, or 60 minutes. Except where noted (*), differences in MSH release were statistically significant ($P < .01$). The percentage of inhibition of MSH release is noted above the bars. Fig. 2 (right). In vitro demonstration of the inhibitory effect of phenylephrine (*PE*) and the stimulating effect of isoproterenol (*ISO*) on MSH secretion from the frog neurointermediate lobe and rat pituitary. The antagonism by isoproterenol of the phenylephrine inhibition is also shown. Each value (mean ± standard error) represents the response of 16 frog skins to MSH released from eight glands under each experimental condition. Significant differences ($P < .01$) were found between the effect of phenylephrine alone compared to that of phenylephrine in the presence of isoproterenol, and between the effects of the two agonists compared to controls.

stimulated MSH release, and this abolished the inhibitory effect of phenylephrine (Fig. 2) (as well as that of NE, E, and DA in other experiments). The inhibitory and stimulatory effects of catecholamines on MSH release in vitro were reversible and could be demonstrated in the frog, the rat, and the mouse. These results suggested that inhibition of MSH release might be controlled through α-adrenergic receptors because release was inhibited by phenylephrine, a specific α-adrenergic agonist (*13*), but not by isoproterenol, a β-adrenergic agonist. This was confirmed in that the α-adrenergic blocking agent Dibenamine (as well as dihydroergotamine in other experiments) antagonized the inhibition of MSH release by E (Fig. 3A). The β-adrenergic blocking agent propranolol blocked the stimulation of MSH release by isoproterenol (Fig. 3B).

Those amines inhibitory to MSH release in medium 199 (Microbiological Associates) were much less effective in either frog Ringer or Krebs Ringer bicarbonate media except when reducing agents such as ascorbic acid and glutathione (each 1 mg/liter) were added. This may explain (*6*) the failure (*14*) of others to demonstrate an inhibitory action of catecholamines on MSH release in vitro. We determined by bioassay (*15*) that catecholamine activity was rapidly lost during incubation in a nonreducing environment.

Electrical activity in the frog pars intermedia, as monitored by microelectrodes (*16*), is affected by photic stimulation of the lateral eyes or pineal body. Since frogs (and many other poikilotherms) rapidly adapt to the albedo of the background environment, ocular photic information is probably conveyed through central nervous system pathways to the hypothalamus and then by further neuronal pathways directly to the pars intermedia cells. Since the actions of the adrenergic agents we studied were blocked by classical pharmacological mechanisms, these catecholamines may exert their effects through α-adrenergic or dopaminergic receptors (or both) of pars intermedia cells. Dopaminergic receptors are considered by some (*17*) to be related to α-adrenergic receptors. Our results do not rule out the possibility that NE, E, and phenylephrine also mediate their effects through α-adrenergic receptors separate from dopaminergic receptors. Both DA- and NE-containing neurons have been demonstrated within the pars intermedia of the rat (*18*), and aminergic neurons apparently innervate the pars intermedia cells of the frog (*19, 20*) and of mice (*21*) and other mammals (*22*).

Although a plexus of catecholamine-containing neurons has not been demonstrated within the vertebrate pars distalis, catecholamines have variable effects on hormone release or inhibition from this organ (*2–10*). It has been reported that NE, E, phenylephrine, and DA inhibit prolactin release in vitro and that both phentolamine and propranolol, used singly or in combination, inhibited NE antagonism of prolactin release, which made it impossible to characterize the supposed adrenergic receptors involved (*5*). In our studies, catecholamines inhibited or enhanced MSH secretion depending on the concentrations employed; since these actions were specifically blocked by recognized pharmacological methods, we do not support the view (*9*) that the direct actions of catecholamines on pituitary function are nonspecific. The contrasting effects of catecholamines on prolactin release are probably most easily explained if lactotrophs, like the cells of the pars intermedia, possess both α- and β-adrenergic receptors. The "biphasic" effects of epinephrine on prolactin release would result, as for MSH release, from β-adrenergic receptor stimulation of prolactin release at low concentrations and a dominant inhibition of prolactin release at higher concentrations. This interpretation is consistent with present pharmacological knowledge of adrenergic receptor mechanisms. The conflicting reports of catecholamine effects on pituitary hormone secretion undoubtably relate to the apparent presence of both inhibitory (α) and stimulatory (β) adrenergic receptors for certain hormone-secreting cells of the pituitary.

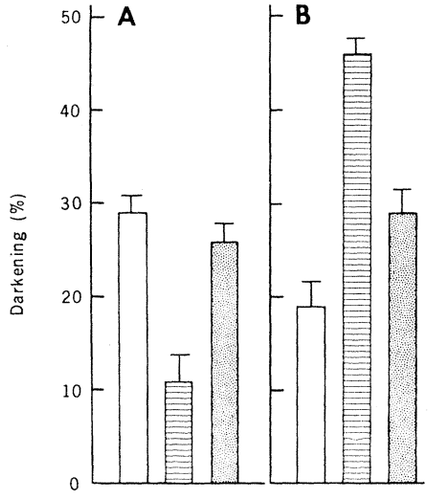

Fig. 3. In vitro effects of adrenergic receptor blockade on epinephrine inhibition and isoproterenol stimulation of MSH release from the rat pituitary. (A) Epinephrine ($10^{-5}M$) was tested alone (cross-hatched bar) and with the α-adrenergic blocking agent Dibenamine ($5 \times 10^{-5}M$) (stippled bar). (B) Isoproterenol ($5 \times 10^{-6}M$) was tested alone (cross-hatched bar) and with the β-receptor blocking agent propranolol ($10^{-5}M$) (stippled bar). In both experiments, values (mean ± standard error) represent the response of eight frog skins to MSH released from four rat pituitaries under each experimental condition; open bars are controls. Significant differences ($P < .01$) were found between MSH secretion in the presence of agonist (epinephrine or isoproterenol) compared to controls, and between secretion in the presence of agonist compared to that in the presence of both agonist and antagonist.

We feel that we have provided the physiological correlate for the morphological evidence of pars intermedia control by direct neuronal innervation. These are, to our knowledge, the first in vitro data that involve possible adrenergic mechanisms in the control of mammalian MSH secretion. Catecholamines have been implicated from in vivo studies to have inhibitory control of MSH release in the rat, but they were proposed to act at the hypothalamic level through the intermediary of a hypothalamic MSH release inhibiting factor (*10*). It was suggested (*23*) that MSH release from the pituitary of the dogfish (*Scyliorhinus canicula*) in vivo is inhibited by amines acting through α-adrenergic receptors at the level of the hypothalamus and pituitary. The present results do not rule out the possibility that adrenergic mechanisms of MSH release inhibition are operative at the hypothalamic level as well as directly on the pars intermedia cells.

Although dopamine and perhaps α-adrenergic receptors may provide the mechanism for the neuronal inhibitory control of MSH release, the precise roles, if any, of β-adrenergic receptors in MSH release mechanisms are unclear. These results contradict the report (*24*) of in vivo studies on MSH release from the frog pars intermedia. The suggestion that β-adrenergic receptors mediate the inhibitory control of MSH release whereas α receptors facilitate MSH release is based, in our opinion, on a failure to appreciate the effects [as local anesthetic (*25*)] of β-adrenergic antagonists on membrane calcium ion flux. Furthermore, assignment of specific adrenergic receptor mechanisms to cellular events should be based on demonstration of agonist in-

hibition by the antagonist, not on the intrinsic activity of the antagonist, from which these interpretations apparently (*24*) were drawn. This MSH release mechanism, in concert with the inhibitory neuronal input, may modulate fine adjustments in MSH secretion. A "doubly innervated secretory unit" (*26*), proposed for regulation of MSH release from the frog pituitary, is supported by both morphological (*20*) and electrophysiological (*16, 26*) evidence. Our results do not rule out the possibility that some cells possess only α-adrenergic receptors whereas others have only β receptors. The relation of adrenergic mechanisms of MSH release control to possible neurosecretory mechanisms (*27*) involving postulated hypothalamic factors inhibiting (*28*) and enhancing (*29*) MSH release is unclear. Evidence for the structure of these possible neurosecretory MSH releasing and inhibiting peptides remains equivocal (*30*).

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Respiration of Benthopelagic Fishes: In situ Measurements at 1230 Meters

Abstract. *The respiration rate in situ of two common benthopelagic fishes,* Coryphaenoides acrolepis *and* Eptatretus deani, *was monitored at 1230 meters in the San Diego Trough. The respiration rate of* C. acrolepis *was two orders of magnitude lower and that of* E. deani *was significantly lower* ($P < .05$) *than rates in comparable shallow-water species.*

Our measurements of the respiration of the rattail *Coryphaenoides acrolepis* and the hagfish *Eptatretus deani* at a depth of 1230 m in the San Diego Trough represent the first successful attempts to determine the metabolic activity in situ of individual deep-sea animals. Previous measurements in situ have shown that the metabolic activities of benthic communities and bacteria are significantly lower in the deep ocean than in shallow water (*1*). Our respiration measurements show that deep-sea fish respire at a significantly lower rate than comparable shallow-water forms.

Both macrourids (rattails) and myxiniids (hagfish) are common benthopelagic fishes in the deep seas of the world oceans. Macrourids are the most abundant group of deep-sea benthic fishes and are predominantly associated with continental slopes (*2*). *Coryphaenoides acrolepis* and *Eptatretus deani* are dominant fish species in the San Diego Trough, along with the sable fish *Anoplopoma fimbria*.

The area of investigation during October 1973 was located 14 miles (26.2 km) off San Diego (32°34.75′N, 117°29.00′W) at a depth of 1230 m. Bottom water temperature was 3.5°C, and the dissolved oxygen concentration was 0.71 ml/liter. Sediments were predominantly clay, and large amounts of fecal pellets were present.

Our work was part of a detailed study of the benthos of the San Diego Trough, in which the remote underwater manipulator (RUM) was used. The RUM is a remote-controlled vehicle with a mechanical arm; it is lowered to the seabed by a conducting cable and is monitored continuously with television cameras. On three successive lowerings we secured a fish trap respirometer to RUM. The respirometer consisted of a Plexiglas box (61 by 30 by 30 cm). A spring-loaded door at one end was designed to be opened and closed with the RUM mechanical arm. A polarographic oxygen electrode (*3*) was inserted in the side of the trap and connected to an amplifier and continuous monitoring recorder which were housed in a glass sphere mounted on the side of the trap. The stirring motor and magnetic stir bar were mounted above the electrode to provide circulation both over the electrode and throughout the fish trap. Approximately 10 g of fresh bonito muscle tissue was enclosed in a wire-mesh box and anchored at the back of

Table 1. Respiration, weight, and length of *Coryphaenoides acrolepis* and *Eptatretus deani*.

Fish	Wet weight (kg)	Overall length (cm)	Respiration (milliliters of oxygen per hour)		Measurement time (min)
			Total	Per kilogram wet weight	
C. acrolepis	1.8	68	4.4	2.4	217
E. deani	0.1	51	0.2	2.2	767