PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 176, 168–174 (1984)

Differential Effects of Adrenergic Agents on Plasma Levels of Immunoreactive β-Endorphin and α-Melanotropin in Rats (41858)

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Abstract. Parallel measurements of plasma α-melanotropin-like immunoreactivity (α-MSH-LI) and β-endorphin-like immunoreactivity (β-END-LI) were used to examine the differential adrenergic control of β-END secretion from the anterior lobe (AL) versus the intermediate lobe (IL) of the rat pituitary gland *in vivo*. Changes in plasma α-MSH-LI levels after treatment with various adrenergic agents served as an index of the secretion of the peptides by the IL. Secretion of β-END-LI from the AL *in vivo* was evaluated using the selective inhibitory effects of dexamethasone on AL release. Inhibition of glucocorticoid synthesis by metyrapone or activation of α-adrenoceptors by clonidine increased ($P < 0.05$) plasma levels of β-END-LI while plasma levels of α-MSH-LI were not affected by either treatment. By contrast, peripherally administered isoproterenol, norepinephrine or epinephrine, each increased plasma levels of α-MSH-LI together with β-END-LI in a dose-dependent manner. The synthetic glucocorticoid, dexamethasone, significantly attenuated the rise in plasma β-END-LI induced by norepinephrine and epinephrine but did not affect the rise in α-MSH-LI. The isoproterenol-induced rises in β-END-LI and α-MSH-LI were inhibited by the β-adrenergic blocker propranolol. By contrast, the α-adrenergic blockers, phentolamine or prazosin, reversed the effect of epinephrine on both peptides while propranolol had no significant effect. The effects of epinephrine, therefore, appear to be α-adrenergic, whereas those of isoproterenol are β-adrenergic. These studies extend earlier findings to indicate that clonidine is a selective activator of AL corticotroph secretion *in vivo*. On the other hand, other adrenergic agents such as norepinephrine, epinephrine, and isoproterenol appear to stimulate secretion from both corticotrophs as well as melanotrophs.

Recent *in vitro* studies from several laboratories have shown that in the rat, β-endorphin (β-END)-related peptides are synthesized, stored, and secreted by cells of both the anterior lobe (AL) and intermediate lobe (IL) of the pituitary gland (1–5). While it has become clear that specific hormones and neurotransmitters differentially affect the release of β-END-like immunoreactivity (β-END-LI) from the two lobes *in vitro* (6–9), the possible independent control of β-END-LI release from the AL versus the IL *in vivo* has been more difficult to study. Since recent studies indicate that in the rat only the AL releases appreciable amounts of opiate-active $\beta\text{-END}_{1\text{-}31}$, whereas the IL secretes primarily inactive forms (i.e., shorter and acetylated β-ENDs) (2, 5), it may be of considerable physiologic importance to recognize the origin of blood-borne β-END-LI released under different conditions.

Several approaches have been considered for evaluating the proportion of blood-borne β-END-LI which originates from the AL or IL. For example, since β-lipotropin (β-LPH), the immunoreactive precursor to β-END, is secreted by the AL, but not the IL *in vitro* (8–11), a stimulus which predominantly increases a form of plasma β-END-LI related to β-LPH (as determined by gel filtration) is thought to indicate largely an AL response (12–14). A second approach is to measure plasma α-melanotropin-like immunoreactivity (α-MSH-LI) which appears to be a specific marker for hormone released from IL melanotrophs. Since α-MSH and β-END peptides are cosynthesized and presumably coreleased from IL melanotrophs (1–5, 15, 16), parallel increases in plasma concentrations of α-MSH-LI and β-END-LI without an associated increase in β-LPH suggests *in vivo* release from the IL. The use of plasma β-LPH and α-MSH as indices of AL and IL secretion, respectively, is further strengthened by a third approach involving the use of a glucocorticoid. *In vitro* experiments demonstrate glucocorticoids, in small amounts, inhibit the release of AL β-END-LI without affecting IL secretion (8, 14). Thus, consistent with the physiologic function

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of glucocorticoids in regulating the pituitary–adrenal axis, dexamethasone may be used to selectively prevent the release of β-END-LI (and adrenocorticotropin) from AL corticotrophs but not β-END-LI (and α-MSH-LI) from IL melanotrophs.

We have previously shown *in vitro* that α- and β-adrenergic stimulation releases β-END-LI selectively from the AL and IL cells, respectively (6). To determine whether α- and β-adrenergic mechanisms exert similar differential control over the secretion of β-END-related peptides from these two lobes *in vivo,* we tested the effects of various adrenergic agents on plasma levels of β-END-LI and α-MSH-LI, the latter being a specific marker for IL secretion. AL secretory activity was determined using glucocorticoid (dexamethasone)-specific inhibition of stimulated plasma β-END-LI levels.

Materials and Methods. Male, Sprague–Dawley rats weighing 150–200 g (Taconic Farms, Inc., Germantown, Pa.) were housed under a 12:12-hr light:dark cycle, having free access to standard rat chow and tap water. The animals were handled once daily for at least 3 days prior to each experiment.

After decapitation, blood was collected into tubes containing 500 μl 10% ethylenediaminetetraacetic acid plus bacitracin (30 μg/ml) and kept on ice. The tubes were centrifuged and plasma was stored at −70°C until assayed.

Plasma β-END-LI was measured by a radioimmunoassay described previously (14, 17). The assay (antiserum C-55) detects β-END (i.e., $\beta\text{-END}_{1\text{-}31}$), purified human β-LPH (gifts of D. Orth, A. Parlow), and several modified forms of β-END (i.e., *N*-acetyl-$\beta\text{-END}_{1\text{-}31}$, *N*-acetyl-$\beta\text{-END}_{1\text{-}27}$, and $\beta\text{-END}_{1\text{-}27}$) on an equimolar basis. Peptides related to the *N*-terminal amino acid sequence of β-END (i.e., leu- and met-enkephalin), however, do not cross-react in the assay. Since the peptides which do cross-react in the present assay are all considered to be synthesized in, and released from, the same pituitary cells as β-END (2, 3, 5, 10), it is likely that changes in total β-END-LI in plasma reflect the secretory activity of these cells.

A new radioimmunoassay for plasma α-MSH was developed. α-MSH (4.5 mg) (Peninsula Labs, San Carlos, Calif.) was conjugated to bovine thyroglobulin (25 mg) (Sigma Chemical Co., St. Louis, Mo.) using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (Bio Rad Labs, Richmond, Calif.) (100 mg) (18) and injected intradermally in an emulsion of equal volumes of Freund's complete and incomplete adjuvants into adult male New Zealand white rabbits (~150 μg α-MSH bound to conjugate/ml/rabbit, Dutchland Laboratory Animals, Inc., Denver, Pa.). Boosting injections were similarly made every 4 weeks and the antiserum chosen (H50) for the assay was collected by ear vein after the fourth administration of conjugate. Radioiodinated α-MSH was prepared as described elsewhere (19) and purified by cation-exchange (SP Sephadex C-25) using slight modifications of Mroz *et al.* (20). Routinely, unextracted plasma was assayed at 100 and 200 μl in duplicate and plasma from hypophysectomized rats which was free of α-MSH-LI was included in the standard curve. The assay (500-μl total volume, 0.5 *M* phosphate buffer, pH 7.4, 0.05% bovine serum albumin, 0.02% sodium azide) of unextracted plasma (50–300 μl) and 1 *N* HCl extracts of rat intermediate lobe produced curves that were parallel to standard α-MSH. Used at a final dilution of 1:250,000 the antiserum specifically bound 35% of the iodinated α-MSH at equilibrium (3 days at 4°C). It should be noted that the inclusion of α-MSH-free plasma in the standard curve increased the effective antibody titer; without plasma a final dilution of 1:100,000 was needed to produce 35% maximum binding at equilibrium. This beneficial effect was due to the reduced nonspecific binding of radiolabeled α-MSH to the glass RIA tubes. Separation of free from bound radioactivity was done by charcoal adsorption and centrifugation (21). The assay, which detects 6–10 pg α-MSH, cross-reacts on an equal molar basis with des-acetyl-α-MSH (Peninsula) and diacetyl-α-MSH (Bachem). It does not, however, detect up to 30 ng/tube of any of the following: deamidated α-MSH, β-MSH, ACTH 1–10, 1–13, or 1–24 (Peninsula), β-END peptides, or β-LPH. Intra- and interassay variation for plasma were 5 and 15%, respectively, for 10 determinations.

The following drugs were used: *l*-epinephrine (Sigma, St. Louis, Mo.), *l*-norepinephrine (Sigma), *l*-isoproterenol (Sigma), clonidine

(Boehringer Ingelheim, Ltd., Ridgefield, Conn.), *d,l*-propranolol (Sigma), phentolamine (Ciba-Geigy, Summit, N.J.), prazosin (Pfizer, Groton, Conn.), dexamethasone (Carter-Glogan Labs, Glendale, Ariz.), metyrapone (Ciba-Geigy).

All values are group means ± SEM. Statistical differences between groups as determined by analysis of variance and Duncan's multiple-range comparison test were considered significant at the $P < 0.05$ level.

Results. *Effects of metyrapone.* Metyrapone (100 mg/kg ip), a glucocorticoid synthesis inhibitor, evoked a brisk rise in plasma β-END-LI, 20 to 60 min after injection (Fig. 1) without affecting plasma α-MSH-LI, suggesting that under conditions of AL stimulation (by removal of negative feedback by corticosterone) α-MSH-LI is not secreted. This supports the concept that plasma α-MSH-LI, which disappears after total hypophysectomy (data not shown), is originating solely from the IL.

Effects of adrenergic agonists and antagonists. Like their effects on plasma levels of β-END-LI (6) the administration of isoproterenol, epinephrine, or norepinephrine all significantly increased plasma levels of α-MSH-LI in a dose-related manner (Fig. 2). The epinephrine- and isoproterenol-induced rises in plasma α-MSH had parallel time courses to those of plasma β-END-LI (6) with peak effects occurring 30 min after injection (Fig. 3). By contrast, clonidine at doses (up to 1 mg/kg) which increase plasma β-END-LI three to fourfold (22), did not influence plasma α-MSH-LI up to 60 min after injection (Fig. 2, Table IV).

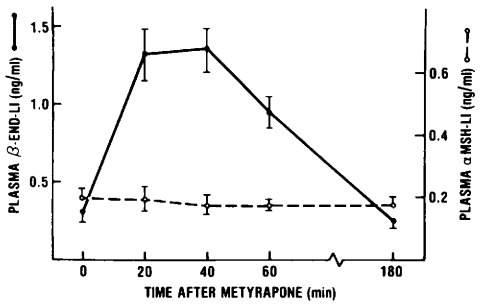

FIG. 1. Comparison of the effects of metyrapone administration on plasma β-END-LI and α-MSH-LI. Rats were injected intraperitoneally with metyrapone (100 mg/kg) or saline (0.9%) and sacrificed at the various time-points after injection. Values are group means ± SEM ($N = 6$ or 7). $P < 0.05$ β-END-LI: 20, 40, 60 min vs 0, 180 min.

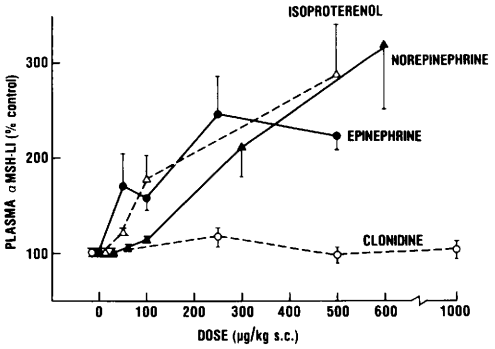

FIG. 2. Dose-related changes in plasma α-MSH-LI after various adrenergic agonists. Rats were injected with the indicated doses of the following drugs (or their vehicles) subcutaneously: isoproterenol, epinephrine, norepinephrine (0.9% saline plus 6 mg% ascorbic acid), clonidine (0.9% saline). The animals were sacrificed 30 min after isoproterenol and epinephrine or 15 min after clonidine. Data are plotted as percentage of control values and represent group means ± SEM ($N = 6$ or 7). Control plasma α-MSH-LI values for each experiment are: isoproterenol 0.22 ± 0.04 ng/ml; epinephrine 0.14 ± 0.02 ng/ml; norepinephrine 0.27 ± 0.02 ng/ml; clonidine 0.35 ± 0.03 ng/ml. $P < 0.05$ vs control: isoproterenol 100, 500 μg/kg; epinephrine 100, 250, 500 μg/kg; norepinephrine 300, 600 μg/kg.

To further characterize adrenergic receptor mechanisms involved in the changes in plasma levels of β-END-LI and α-MSH-LI after the various agonists, the effects of several α- and β-adrenergic blockers were investigated. The administration of the β-adrenergic blocker, propranolol (5 mg/kg ip), 30 min after isoproterenol (300 μg/kg sc) significantly attenuated the drug-induced rise in plasma β-END-LI (Table I) and fully blocked the increase in plasma α-MSH-LI (Table I). In contrast, propranolol (5 mg/kg ip), did not significantly influence the epinephrine-induced rise in plasma β-END-LI or α-MSH-LI (Table II and III). Pretreatment with the α-adrenergic antagonist, phentolamine (5 mg/kg ip), however, significantly inhibited the epinephrine-induced rise in both peptides (Table II). Similar antagonism of the epinephrine effect on plasma α-MSH-LI and β-END-LI was obtained by prior administration of another α-adrenergic blocker, prazosin (600 μg/kg ip,

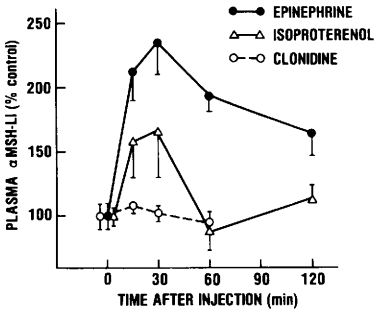

FIG. 3. Time-related changes in plasma α-MSH-LI after epinephrine, isoproterenol, or clonidine. Rats were administered either epinephrine (100 μg/kg), isoproterenol (200 μg/kg), or their vehicle (0.9% saline plus 6 mg% ascorbic acid) or clonidine (500 μg/kg) or its vehicle (0.9% saline), subcutaneously, and sacrificed at the indicated time points after injection. Data are plotted as percentage of control values and represent group means ± SEM. Control plasma α-MSH-LI values for each experiment are: epinephrine 0.21 ± 0.2 ng/ml; isoproterenol 0.21 ± 0.01 ng/ml, clonidine 0.28 ± 0.03 ng/ml. $P < 0.05$ vs control: epinephrine 15, 30, 60, 120 min; isoproterenol 15, 30 min.

Table III). The clonidine-induced rise in plasma β-END-LI also appears to be α-adrenergic because pretreatment with prazosin blocked its effect as well (Table IV).

Effects of dexamethasone. The administration of dexamethasone (60 μg/kg ip, 4 hr) significantly attenuated the rise in plasma β-END-LI elicited by norepinephrine (400 μg/kg sc, 30 min) and epinephrine (250 μg/kg sc, 30 min) (Fig. 4). The two- to threefold rise in plasma α-MSH-LI induced by both agents was not significantly affected by dexamethasone.

Discussion. These studies demonstrate that various adrenergic agonists evoke the release of β-END-LI and related peptides differentially from cells of the AL (i.e., corticotrophs) versus cells of the IL (i.e., melanotrophs) *in vivo*. For

TABLE I. BLOCKADE BY PROPRANOLOL OF ISOPROTERENOL-INDUCED INCREASE IN PLASMA β-END-LI AND α-MSH-LI

Treatment	Plasma immunoreactivity (ng/ml) β-END-LI	α-MSH-LI
SAL + SAL	0.22 ± 0.02	0.22 ± 0.02
PROP + SAL	0.25 ± 0.05	0.21 ± 0.03
SAL + ISOPROT	1.50 ± 0.11[a]	0.33 ± 0.02[a]
PROP + ISOPROT	0.88 ± 0.10[b]	0.24 ± 0.02

Note. Rats were injected with either 0.9% saline (SAL) or propranolol (PROP, 5 mg/kg) intraperitoneally. Thirty minutes later they received either vehicle (0.9% saline containing 6 mg% ascorbic acid) or isoproterenol (ISOPROT) (300 μg/kg sc) and were sacrificed 30 min later. Values are group means ± SEM ($N = 6$ or 7).

[a] $P < 0.01$ vs all other groups.

[b] $P < 0.05$ vs SAL + SAL and PRO + SAL.

TABLE II. EFFECTS OF PHENTOLAMINE AND PROPRANOLOL ON EPINEPHRINE-INDUCED RISE IN PLASMA β-END-LI AND α-MSH-LI

Treatment	Plasma immunoreactivity (ng/ml) β-END-LI	α-MSH-LI
SAL + SAL	0.16 ± 0.01	0.21 ± 0.01
PHENTOL + SAL	0.27 ± 0.05	0.19 ± 0.02
PROP + SAL	0.17 ± 0.01	0.24 ± 0.02
SAL + EPI	1.42 ± 0.09[a]	0.83 ± 0.12[a]
PHENTOL + EPI	0.50 ± 0.08[b]	0.24 ± 0.02
PROP + EPI	1.32 ± 0.14[a]	1.18 ± 0.13[a]

Note. Rats were injected with either 0.9% saline (SAL), phentolamine (PHENTOL, 5 mg/kg ip), or propranolol (PROP, 5 mg/kg ip). Thirty minutes later they received either vehicle (0.9% saline containing 6 mg% ascorbic acid) or epinephrine (EPI, 200 μg/kg sc) and were sacrificed 30 min thereafter. Values represent group means ± SEM ($N = 6$ or 7).

[a] $P < 0.01$ vs all other groups.

[b] $P < 0.01$ vs SAL + SAL.

TABLE III. BLOCKADE BY PRAZOSIN OF EPINEPHRINE-INDUCED RISE IN PLASMA β-END-LI AND α-MSH-LI

Treatment	Plasma immunoreactivity (ng/ml) β-END-LI	α-MSH-LI
SAL + SAL	0.27 ± 0.03	0.43 ± 0.05
PRAZ + SAL	0.34 ± 0.05	0.40 ± 0.03
PROP + SAL	0.22 ± 0.03	0.42 ± 0.07
SAL + EPI	2.29 ± 0.24[a]	0.79 ± 0.13[a]
PRAZ + EPI	0.50 ± 0.14	0.42 ± 0.03
PROP + EPI	1.60 ± 0.21[b]	0.91 ± 0.14[b]

Note. Rats received either 0.9% saline (SAL), prazosin (PRAZ, 600 μg/kg), or propranolol (PROP, 5 mg/kg) intraperitoneally 30 min before either vehicle (0.9% saline plus 6 mg% ascorbic acid) or epinephrine (EPI, 150 μg/kg) subcutaneously; the animals were sacrificed 30 min later. Values are group means ± SEM ($N = 6$ or 7).

[a] $P < 0.01$ vs all other groups.

[b] $P < 0.01$ vs all other groups except SAL + EPI.

TABLE IV. BLOCKADE BY PRAZOSIN OF CLONIDINE-INDUCED INCREASE IN PLASMA β-END-LI

Treatment	Plasma immunoreactivity (ng/ml)	
	β-END-LI	α-MSH-LI
SAL + SAL	0.47 ± 0.06	0.32 ± 0.01
PRAZ + SAL	0.47 ± 0.07	0.32 ± 0.03
SAL + CLON	1.16 ± 0.24[a]	0.30 ± 0.02
PRAZ + CLON	0.62 ± 0.04	0.31 ± 0.02

Note. Rats were injected with either saline (SAL, 0.9%) or prazosin (PRAZ, 600 μg/kg) intraperitoneally followed 30 min later by a second injection of saline (0.9%) or clonidine (CLON, 500 μg/kg) subcutaneously; the animals were sacrificed 30 min later. Values are group means ± SEM (N = 6 or 7).

[a] $P < 0.01$ vs all other groups.

example, the administration of clonidine, while increasing plasma levels of β-END-LI, has no effect on plasma α-MSH-LI indicating that IL melanotrophs probably do not contribute to the plasma β-END-LI response following clonidine. This interpretation is strengthened by previous studies in which clonidine was shown to stimulate β-END-LI release from AL cells but not IL cells *in vitro* (11). Further, the rise in plasma β-END-LI evoked by clonidine was fully inhibited a dose of dexamethasone which does not affect IL secretion ((14), Fig. 4). The failure of clonidine to alter plasma levels of α-MSH-LI in the present study, therefore, is consistent with α-MSH-LI being a specific marker of IL function. This approach which has recently been utilized elsewhere (15, 16) is further supported by the ineffectiveness of metyrapone or dexamethasone to alter plasma levels of α-MSH-LI (see Figs. 1, 4).

Among α-adrenergic agents, clonidine is unique in its ability to selectively stimulate β-END-LI release from the AL without altering IL secretion. This point is emphasized by both the present findings with norepinephrine and epinephrine, as well as by other findings (unpublished) with adrenergic agents such as α-methylnorepinephrine and phenylephrine (0.3–1 mg/kg, ip) all of which evoke dose-dependent, parallel increases in plasma β-END-LI and α-MSH-LI. Moreover, dexamethasone pretreatment completely prevents the plasma β-END-LI response to clonidine (14) but only partially inhibits β-END-LI release evoked by mixed AL and IL stimulating agents (e.g., norepinephrine and epinephrine, Fig. 4). Consistent with the view that glucocorticoids selectively inhibit AL secretion *in vivo*, under the present conditions dexamethasone fails to influence the rise in plasma α-MSH-LI following norepinephrine or epinephrine injection (Fig. 4).

Although the mechanisms by which clonidine increases pituitary β-END-LI release is mediated via an α-, but not β-adrenergic receptor, the specific α-adrenoceptor subtype involved is not readily defined *in vivo*. We have shown that the α_2-adrenergic antagonist, yohimbine, as well as other adrenergic blockers with mixed α_2/α_1 activity (i.e., phentolamine, phenoxybenzamine) blocked the effects of clonidine on β-END-LI (21), findings which are consistent with the predominant action of clonidine on α_2-adrenoceptors (22). Unexpectedly, however, in the present study we observed that the specific α_1-antagonist, prazosin, also fully blocked clonidine's effect on plasma β-END-LI. Although it is conceivable that at the doses used here (500 μg/kg ip) clon-

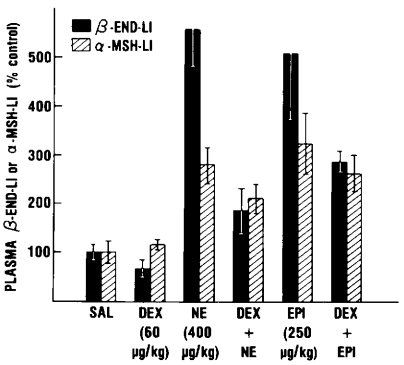

FIG. 4. Blockade of norepinephrine (NE)- and epinephrine (EPI)-induced increases in plasma β-END-LI by dexamethasone. Rats received dexamethasone (DEX) (60 μg/kg ip) or its vehicle (0.9% saline (SAL)) 4 hr before norepinephrine (400 μg/kg) or epinephrine (250 μg/kg) or their vehicle (0.9% saline plus 6 mg% ascorbic acid). Data are group means ± SEM represented as percentage control levels (N = 6 or 7). Control plasma values are: β-END-LI 0.25 ± 0.04 ng/ml; α-MSH-LI 0.13 ± 0.03 ng/ml. $P < 0.05$ β-END-LI: SAL vs NE, EPI, DEX + EPI; EPI, NE vs DEX + EPI, DEX + NE; α-MSH-LI: SAL vs NE, EPI, DEX + NE, DEX + EPI.

idine exhibits significant α_1 activity, this does not appear to be the case since clonidine failed to stimulate the release of α-MSH-LI in a manner similar to that observed for norepinephrine and epinephrine (Tables II and III, Figs. 2 and 4). One would anticipate that clonidine would have access to the same α-adrenergic sites which mediate α-MSH-LI release in response to the catecholamines. It is possible, however, that the adrenoceptor regulating the pituitary β-END-LI response to clonidine is also capable of interacting with prazosin. *In vitro* data indicate that this may be the case for the adrenoceptor located directly on the corticotroph cell (23). Alternatively, multiple counteractive actions of clonidine in the brain and/or periphery could result in a condition under which no net change in α-MSH-LI release occurs.

The present findings that the α-adrenergic antagonists phentolamine and prazosin inhibit the stimulatory effect of epinephrine on plasma β-END-LI are in agreement with our earlier observations using phenoxybenzamine (6). The inhibition of the epinephrine-induced rise in plasma α-MSH-LI by α-adrenergic blocking drugs but not by propranolol (Tables II, III), however, was unexpected because the bulk of evidence indicates that a β-adrenergic mechanism stimulates IL secretion *in vitro* and *in vivo* (9, 15, 25–27). Indeed, the attenuation by propranolol of the increase in plasma β-END-LI and α-MSH-LI observed after the β-adrenergic agonist, isoproterenol (Table I) reinforces this possibility. It is unlikely, that epinephrine is directly stimulating α-adrenergic mechanisms on melanotrophs after its intraperitoneal administration because *in vitro* findings indicate that epinephrine acts solely as β-adrenergic agonist on IL cells in culture (6). Therefore, under the present conditions it seems likely that peripheral administration of epinephrine evokes α-MSH-LI release through actions within the brain. The circumventricular organs do exhibit incomplete blood–brain barriers and thus provide sites at which even polar molecules like epinephrine can gain entry into the central nervous system. Accordingly, the α-adrenergic receptor which mediates α-MSH-LI release in response to injected epinephrine may reside within the brain although unknown peripheral mechanisms and/or a generalized sympathetic response may also mediate the increase in IL secretion. Similar consideration must be taken in interpreting the results obtained with the other centrally active agents administered peripherally.

In summary, α-adrenergic stimulation by peripherally administered epinephrine enhances release of peptides *in vivo* from both corticotrophs and melanotrophs of the rat pituitary. In addition, the release of peptides from melanotrophs is also activated by isoproterenol through β-adrenergic receptors. The present data, however, do not rule out that a portion of the plasma β-END-LI stimulated by isoproterenol arises also from corticotrophs of the AL; it has been reported recently (13) that dexamethasone pretreatment antagonizes isoproterenol-induced stimulation of plasma β-END-LI. The present results showing that clonidine administration does not influence plasma α-MSH-LI extends our previous findings suggesting that this agent is a specific stimulator of β-END-LI from the AL *in vivo* (14).

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Received September 26, 1983. P.S.E.B.M. 1984, Vol. 176.
Accepted February 24, 1984.