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## Melanophore Stimulating Hormone: Release Inhibition by Ring Structures of Neurohypophysial Hormones


Abstract. *Tocinamide and tocinoic acid, ring structures of oxytocin, are potent inhibitors of the release of melanophore stimulating hormone from the rat and hamster pituitary in vitro. Tocinamide is effective at concentrations as low as* $10^{-14}$M *on the mammalian pituitary. These peptides do not affect release of the hormone on the frog* (Rana pipiens) *pars intermedia, but they do inhibit release in the bullfrog* (Rana catesbeiana) *and the toad* (Bufo marinus). *The specificity of the peptides on inhibition of the hormone is demonstrated by the fact that oxytocin, lysine vasopressin, and pressinoic acid and pressinamide (ring structures of the vasopressins) do not show such inhibitory activity. Hypothalamic extracts of either the frog* (Rana pipiens) *or the rat inhibit release of the hormone from pituitaries of either species. The inhibitory effects of tocinamide and tocinoic acid, like that of hypothalamic extracts, are reversible.*


The release of melanophore stimulating hormone (MSH) from the vertebrate pars intermedia can be inhibited by the hypothalamus (*1*). Direct neurosecretory (*2*) and an adrenergic (*3*) innervation of pars intermedia cells have been suggested as the morphological basis for this inhibitory control of hormone release. It has been suggested that both inhibitory (*4*) and stimulatory (releasing) (*5*) factors of hypothalamic origin regulate pars intermedia function. The chemical structure of an MSH release inhibiting factor (MRIF) has been reported (*6*) to be L-Pro-L-Leu-Gly-$NH_2$, the side chain of oxytocin. We have found (*7*) that this synthetic crystalline tripeptide failed to inhibit the in vitro release of MSH from either rat or frog pituitaries, whereas hypothalamic extracts of these species were effective inhibitors of MSH release. However, we have found that tocinoic acid (*8*)

L-Cys-L-Tyr-L-Ile-L-Gln-L-Asn-L-Cys-OH

the ring of oxytocin (*9*), is a potent inhibitor of MSH release from the rat pars intermedia, and we have proposed that it (or a closely related structure) may be a more likely candidate as the natural MRIF in vertebrates. We now provide additional data on the MRIF-like activity of various ring compounds related to the neurohypophysial hormones in a number of vertebrate species.

Rats (Sprague-Dawley), hamsters (*Mesocricetus auratus*), toads (*Bufo marinus*), and frogs [*Rana pipiens* and *Rana catesbeiana* (bullfrog)] were used. The amount of MSH released into the incubation medium from isolated pituitaries or pars intermedia is determined (*10*) by photoreflectance methods described originally for the frog skin bioassay for MSH (*11*). The MRIF-like activity of various neurohypophysial hormone structures is reported as the percentage of inhibition of MSH release, compared to the control release taken as 100 percent, or in some cases, simply as the percentage of darkening. Student's *t*-test was used to determine statistical significance in all experiments.

Tocinoic acid inhibits MSH release from the rat pituitary at concentrations as low as $10^{-10}$ g/ml (*7*). We now report that tocinamide (the ring structure of oxytocin terminating in a carboxamide group)

L-Cys-L-Tyr-L-Ile-L-Gln-L-Asn-L-Cys-$NH_2$

inhibits MSH release at even lower concentrations (Fig. 1). In the mammal pituitary, this compound is about equally effective at any of the various concentrations employed. However, in the hamster pituitary, tocinamide at equivalent doses is less effective in inhibiting MSH release (Table 1). Both the amide and the acid inhibit MSH release from the toad (*Bufo marinus*) pituitary and are variable in their MRIF-like activity on the bullfrog pituitary. In *Rana pipiens*, neither the acid, as reported previously (*7*), nor the amide inhibits MSH release (Table 1).

The specificity of tocinamide and tocinoic acid inhibition of MSH release from the mammalian pituitary is demonstrated by the fact that we have found that neither oxytocin, lysine vasopressin, nor pressinoic acid

L-Cys-L-Tyr-L-Phe-L-Gln-L-Asn-L-Cys-OH

or its amide, pressinamide—the ring structures of the vasopressins (*12*)—possesses any MRIF-like activity in vitro. These structures are similarly ineffective in preventing MSH release from

Table 1. Demonstration in vitro of the MRIF-like activity of tocinamide and tocinoic acid. Twelve pituitaries (hamster) or four pars intermedia (amphibian) were incubated at each concentration. Each value represents the percentage inhibition of MSH release as compared to the control release taken as 100 percent; N.S., not significant. These results are representative of numerous experiments which have provided similar data.

| Tocinamide | | | Tocinoic acid | | |
|---|---|---|---|---|---|
| Concentration (g/ml) | Inhibition of control (%) | *P* | Concentration (g/ml) | Inhibition of control (%) | *P* |
| | | Hamster | | | |
| $10^{-8}$ | 33 ± 3.1 | < .01 | $10^{-5}$ | 36 ± 2.8 | < .01 |
| $10^{-10}$ | 30 ± 3.0 | < .01 | $10^{-6}$ | 32 ± 3.6 | < .01 |
| $12^{-12}$ | 24 ± 2.9 | < .05 | | | |
| | | *Bufo marinus* | | | |
| $10^{-7}$ | 41 ± 2.5 | < .001 | $10^{-5}$ | 37 ± 2.4 | < .01 |
| $10^{-9}$ | 27 ± 3.4 | < .05 | $10^{-6}$ | 31 ± 1.7 | < .01 |
| $10^{-11}$ | 25 ± 2.9 | < .05 | | | |
| | | *Rana catesbeiana* | | | |
| $10^{-5}$ | 70 ± 1.6 | < .001 | $10^{-6}$ | 45 ± 2.5 | < .01 |
| $10^{-6}$ | 20 ± 4.6 | N.S. | $10^{-8}$ | 0 ± 2.4* | N.S. |
| $10^{-9}$ | 13 ± 3.3 | N.S. | | | |
| | | *Rana pipiens* | | | |
| $10^{-5}$ | 0 ± 2.1* | N.S. | $10^{-5}$ | 0 ± 2.8 | N.S. |
| $10^{-7}$ | 0 ± 1.7 | N.S. | $10^{-6}$ | 0 ± 1.8 | N.S. |

* Zero inhibition represents experimental groups where release of MSH was equal to or greater than that of the control.

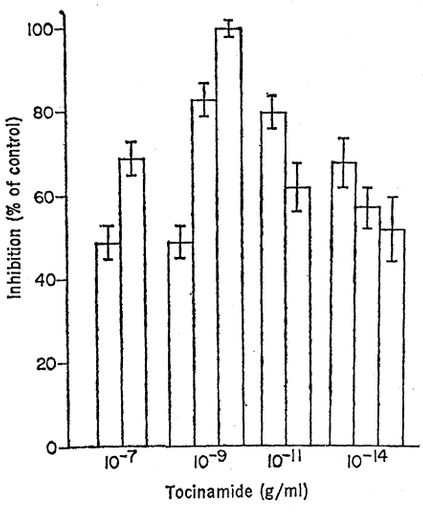


Fig. 1. Demonstration of the MRIF-like activity of tocinamide on the rat pituitary in vitro. Values represent the mean of the percentage inhibition of MSH release (± S.E.); eight frog skins were used for the bioassay. Each bar represents data from four pituitaries. All $P$ values are statistically significant ($P < .001$).

amphibian pars intermedia in vitro. Therefore, these latter compounds cannot be considered as candidates for the possible vertebrate MRIF.

Although both vasotocin and mesotocin are considered to be the neurohypophysial hormones of amphibians and reptiles, their ring structures are the same as that found in oxytocin. The only remaining naturally occurring vertebrate neurohypophysial hormones known are isotocin (present in teleosts) and glumitocin (present in elasmobranchs). Their related cyclic compounds, [4-serine]tocinoic acid

L-Cys-L-Tyr-L-Ile-L-Ser-L-Asn-L-Cys-OH

and the corresponding amide, apparently have no MRIF-like activity (preliminary results).

Our results might suggest that either tocinamide or tocinoic acid, the ring structures of the neurohypophysial hormone oxytocin, is the mammalian MRIF, and that perhaps a closely related structure is the amphibian MRIF. Certainly the concentration at which tocinamide is active in the animals tested is similar to, or less than, that found for other hormones (*13*) or hypothalamic releasing factors (*14*). That there is more than one MRIF regulating pars intermedia function has not previously been suggested. However, such a suggestion appears to be inconsistent with our observations (Fig. 2A) that rat hypothalamic extracts will inhibit MSH release in vitro from both rat and frog (*Rana pipiens*) pituitaries and that frog (*Rana pipiens*) hypothalamic extracts are equally effective in inhibiting MSH release from rat or frog (*Rana pipiens*) pituitaries in vitro. The inhibition by neurohypophysial ring structures, like that of hypothalamic extracts, is reversible since MSH release is at least equal to (or greater than) that of control pituitaries when the glands are returned to media lacking the inhibitor (Fig. 2B).

In summary, these results show that tocinoic acid and especially tocinamide are remarkably active compounds at inhibiting the release of MSH from mammalian (rat and hamster) pituitaries in vitro, and are quite active on some amphibian pituitaries (*Bufo marinus* and *Rana catesbeiana*) in vitro, but not on others (*Rana pipiens*). Although these results might suggest the possibility of more than one vertebrate MRIF, this suggestion is apparently contradicted by the observation that hypothalamic extracts from amphibians are as effective in inhibiting MSH release from either the amphibian or the mammalian pituitary as are extracts from mammalian species. These latter results suggest the presence of a common vertebrate MRIF, and thus leave unclarified the nature of this inhibiting factor.

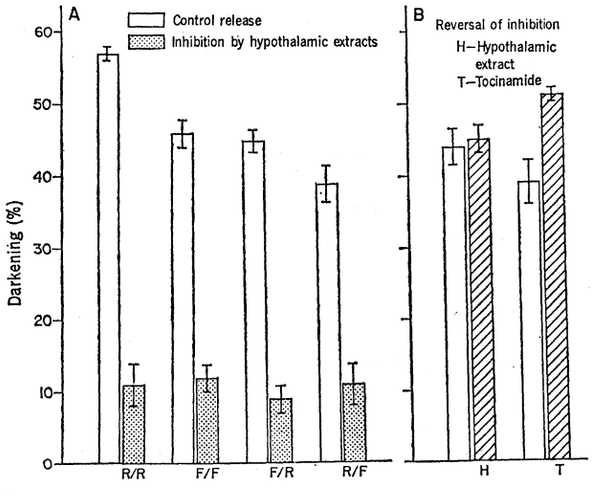


Fig 2. (A) In vitro demonstration of MSH release inhibition by hypothalamic extracts. Hypothalamic extracts (three hypothalami per pituitary) of rat ($R$) and frog ($F$) (*R. pipiens*) inhibited MSH release from pituitaries of the same species and of the other species. $R/R$, rat on rat; $F/F$, frog on frog; $F/R$, frog on rat; $R/F$, rat on frog. (B) Pituitaries inhibited by hypothalamic ($H$) extracts or by tocinamide ($T$) release MSH when the glands are returned to normal media. Each value represents the mean darkening response (± S.E.) of eight skins to the MSH released under each experimental condition. Differences were statistically significant ($P < .001$).


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10. The abbreviations for the amino acid residues are: Tyr, tyrosine; Ile, isoleucine; Gln, glutamine; Asn, asparagine; Ser, serine; and Phe, phenylalanine; Cys, half-cystine.
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