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NEW FUNCTIONS OF CLASSICAL HORMONE, α-MELANOPHORE-STIMULATING HORMONE

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Abstract It is well-known that α-melanophore-stimulating hormone (α-MSH) release from the amphibian pars intermedia (PI) depends on the light condition of the animal's background. In the present study, we present two new functions of α-MSH in amphibians and mammals. In *Xenopus laevis*, we show that temperatures below 8 ℃ stimulate α-MSH secretion of the PI and skin darkening, with a maximum at 5 ℃, under regulation of the hypothalamus rule, independently from the illumination state of the background. The cold-induced α-MSH release of the PI was inhibited by neuropeptide-Y-producing suprachiasmatic-melanotrope-inhibiting neurons in the ventrolateral area of the suprachiasmatic nucleus but increasely in thyrotropin-releasing-hormene-containing neurons of the magnocellular nucleus. It is known that intracerebroventricular (ICV) administration of a low dose of interleukin-1β (IL-1β) induces hyperalgesia in rat and that this effect can be inhibited by α-MSH. To identify the part of the brain that is affected by hyperalgesia-inducing IL-1β and the site of α-MSH concerned, we have examined Fos expression in the brain in response to ICV microinjection of α-MSH and/or IL-1β. Following injection of 10 pg IL-1β, hyperalgesia was induced and Fos became expressed in the paraventricular nucleus (PVN) of the hypothalamus and in the arcuate nucleus (ARC), which contains α-MSH-producing neurons. ICV co-injection of IL-1β with 30 ng α-MSH fully inhibited both hyperalgesia and Fos expression in the PVN and the ARC. We conclude that PVN neurons are activated by hyperalgesic IL-1β and propose that this effect is abolished by α-MSH released from the ARC but not from the pituitary gland.

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Key words: α-melanophore-stimulating hormone (α-MSH); low temperature; Fos; hyperalgesia; interleukin-1β (IL-1β)

Introduction

In this review, we summarize two studies on new roles of classical hormone α-MSH in amphibians and mammals. It is well-known that α-melanophore-stimulating hormone (α-MSH) is released from the neuroendocrine melanotrope cells in the pars intermedia (PI) of the amphibian pituitary gland and regulates the dispersion of melanin in skin melanophores during the process of skin color adaptation to the light condition of the background[1-3]. This process is neurally regulated by several neurochemical messengers originating in the brain and by autocrine factors produced by the melanotropes, as was shown in much detail especially in the toad *Xenopus laevis*, the frog *Rana ridibunda* and *Rana nigromaculata*[1-17]. In addition to light, low temperature seems to influence amphibian skin color but the role of temperature in the functioning of the PI is poorly understood[18,19]. Firstly as a new function of α-MSH, we present detailed information on the

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effects of low temperature on skin color adaptation and on α-MSH release from the PI of *Xenopus laevis*. Also, we report on regulatory system that is responsible for the low temperature-induced α-MSH release from the PI, using double fluorescence immunohistochemistry for Fos as an indicator for neural and cellular activity[20,21].

In mammals, α-MSH-containing neurons, nerve fibers and cells exist in several parts of the brain and in the PI[22-24] whereas α-MSH does not play an important role in skin color control. These facts are full of interesting suggestions on new functions of α-MSH in mammals. Secondly we present a role of α-MSH as host defense modulator in rat, that is, inhibitory effects of α-MSH on interleukin-1β-induced hyperalgesia and neural activation in the paraventricular nucleus (PVN) and the arcuate nucleus (ARC) of the rat, using Fos immunohistochemistry.

1. Low temperature stimulates α-MSH secretion and inhibits background adaptation in *Xenopus laevis* [Refer to 25 for details]

In the present study, we carried out nine experiments on the effect of low temperature on this skin adaptation process in the toad *Xenopus laevis*, using the skin melanophore index (MI) bioassay and an α-MSH radioimmunoassay (RIA) to measure skin color adaptation and α-MSH secretion respectively[17,26,27]. Although measuring the MI does not provide direct data on the concentration of α-MSH in the blood, there is a direct and log-linear relationship between the MI and the α-MSH plasma content as measured by RIA[28]. Compared with *in vitro* RIA, the *in vivo* MI bioassay has prominent features in *Xenopus laevis*, that is, the relative MSH contents in same animal can be measured easily and continually in conventional laboratory condition[17,26-29]. We show that temperatures below 8℃ stimulate α-MSH secretion and skin darkening, with a maximum at 5℃,

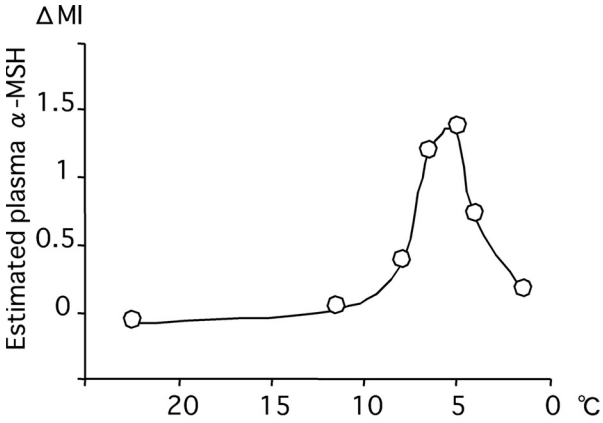

Figure 1 α-MSH release from the pars intermedia (PI) of *Xenopus laevis* in a cold environment were estimated by ΔMIs, after 3 h stay at various temperatures. ΔMIs by the *in vivo* MI bioassay reflect plasma α-MSH concentrations.

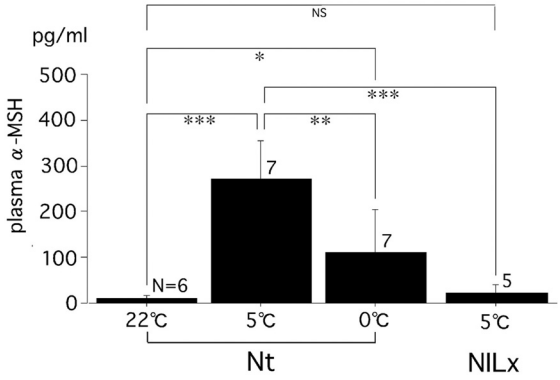

Figure 2 Plasma α-MSH concentrations of nontreated (Nt) and NIL-extirpated (NILx) toads on a white background, after 3 h stay at different temperatures (22℃, 5℃, 0℃), measured by RIA. Means±SD. *P<0.05, **P<0.01, ***P<0.001. NS no significant difference. Number of animals (N) is indicated on top of each column.

independently from the illumination state of the background (Figure 1).

No significant stimulatory effect of low temperature on the MI and α-MSH plasma contents was noted when the experiment was repeated with toads from which the neurointermediate lob (NIL) had been surgically extirpated (Figure 2).

This indicates that low temperature stimulates α-MSH release from melanotrope cells located

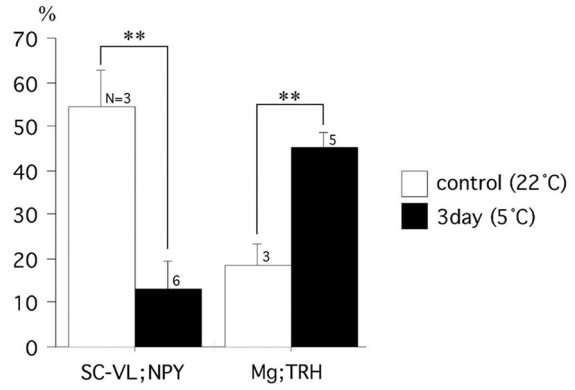

Figure 3 Percental changes in Fos-immunoreactivity of NPY-containing neurons in ventrolateral area of SC (SC-VL) and of TRH-containing neurons in Mg, at control temperature (22℃) and after 3 days of cold exposure (5℃). Means±SD. **P<0.01. NS not significant. Number of animals (N) is indicated on top of each column.

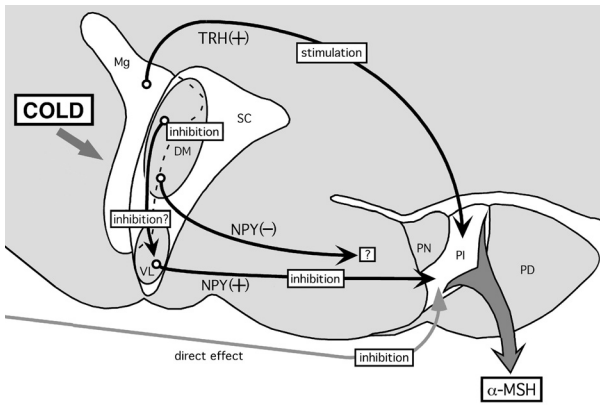

Figure 4 Hypothetic scheme of the regulation of α-MSH release from melanotrope cells in the pars intermedia (PI) of *Xenopus laevis* in a cold environment, via TRH and NPY, in a sagittal plane through the brain and pituitary gland. DM; dorsomedial area of SC, Mg; magnocellular nucleus, PD; pars distalis, PI; pars intermedia, PN; pars nervosa, SC; suprachiasmatic nucleus, VL; ventrolateral area of SC.

in the PI. An *in vitro* superfusion study with the NIL demonstrated that low temperature does not act directly on the PI[25,30]. A possible role of the central nervous system in cold-induced α-MSH release from the PI was tested by studying the hypothalamic expression of Fos and the coexistence of Fos with the regulators of melanotrope cell activity, neuropeptide Y (NPY) and thyrotropin-releasing hormone (TRH), using double fluorescence immunohistochemistry. Upon lowering temperature from 22 to 5 ℃, in white-adapted animals Fos expression decreased in NPY-producing suprachiasmatic-melanotrope-inhibiting neurons (SMIN) in the ventrolateral area of the suprachiasmatic nucleus (SC) but increased in TRH-containing neurons of the magnocellular nucleus (Mg) (Figure 3).

We conclude that temperatures around 5 ℃ inactivate the SMIN in the SC and activate TRH-neurons in the Mg, resulting in enhanced α-MSH secretion from the PI, darkening the skin of white-adapted *Xenopus laevis* (Figure 4).

2. α-MSH antagonizes interleukin-1β-induced hyperalgesia and Fos-expression in the paraventricular and arcuate nucleus of the rat [Refer to 31 for details]

Interleukin-1β (IL-1β) plays an important role in immunoregulation and also mediates non-specific host-defense responses such as fever and sensory response distortion[32-34]. In rat, intracerebroventricularly (ICV) administered IL-1β induces hyperalgesia when given in a low, non-pyrogenic concentration (10 pg/kg to 1 ng/kg), and fever in a higher, non-hyperalgesic concentration (100 ng/kg)[35-37]. ICV administration of a pyrogenic dose of IL-1β also stimulates the expression of the Fos protein, a marker for cellular activity[21], in neurons of the paraventricular nucleus (PVN) of the hypothalamus[38-43], suggesting that pyrogenic IL-1β activates PVN neurons. However, the target of hyperalgesia-inducing IL-1β in the central nervous system (CNS) is unknown. While, from physiological experiments on rats, it has been

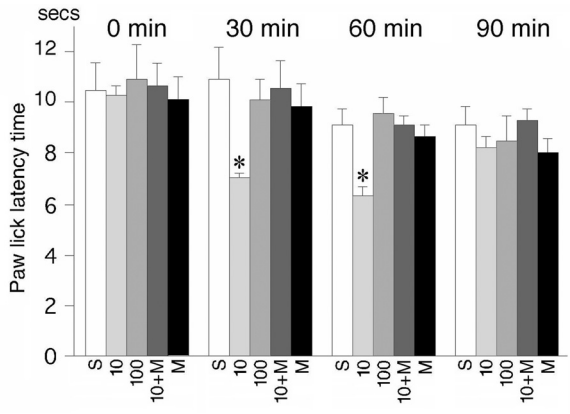

Figure 5 Paw lick latency time in hot-plate test, 0, 30, 60 and 90 min after ICV-microinjection with saline (S; N=5), 10 pg/animal IL-1β (10; N=5), 100 ng/animal IL-1β (100; N=6), 10 pg/animal IL-1β+30 ng/animal α-MSH (10+M; N=6) and 30 ng/animal α-MSH (M; N=5). Means± S.E.M. Asterisks indicate significant difference with each of the four other groups, at P≤0.05.

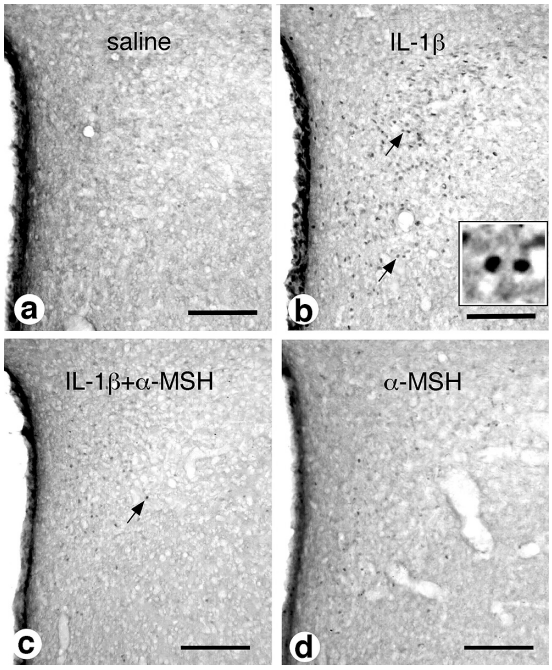

Figure 6 Fos-positive neuronal nuclei (arrows) in the total PVN, 90 min after ICV-administration of saline (a), 10 pg/animal IL-1β (b; IL-1β), 10 pg/animal IL-1β+30 ng/animal α-MSH (c; IL-1β+α-MSH) or 30 ng/animal α-MSH (d; α-MSH). Inset in b shows detail of Fos-positive nuclei. Scale bar=100μm.

concluded that α-MSH inhibits hyperalgesia induced by ICV injection of IL-1β, but the site of α-MSH release concerned is not known.

Here, we study the possible involvement of pituitary α-MSH and of α-MSH present in the brain[22-24] in the antagonism of hyperalgesic IL-1β in rat, focusing on Fos expression in the PVN, in the arcuate nucleus (ARC), containing α-MSH neurons, and in the α-MSH-producing melanotrope cells in the PI.

Materials and Methods

Adult male Wister rats (age 8-16 weeks; 250-330 g) implanted a stainless steel guide cannula into the left lateral cerebral ventricle (LCV) for microinjection of α-MSH and/or IL-1β, were housed under standard laboratory conditions with food and water freely available. The effects of drug injection on algesia were determined using a hot-plate test to estimate the degree of sensory distortion as a measure for hyperalgesia[35-37,44,45]. A rat was placed on the plate, heated to 51.5±0.5 ℃, and the time until the first avoidance response (paw lick latency time) was determined. The target of hyperalgesia-inducing IL-1β was investigated by immunohistochemistry for Fos (ABC-DAB method) as an indicator for cellular activity. The number of Fos-immunoactive neurons or cells in the PVN, ARC and PI were counted using Nikon drawing tube.

Results and Discussion

Hyperalgesia induced by IL-1β (Figure 5)

ICV microinjection of a low dose of IL-1β (10 pg/animal) induces hyperalgesia for a noxious heat stimulus and co-injected α-MSH completely abolishes this response. Also, injection of the high dose of 100 ng/animal IL-1β does not induce hyperalgesia.

Fos expression in the PVN (Fig 6, 7, 8)

After injecting such a hyperalgesic dose of IL-1β, the number of Fos-immunoreactive

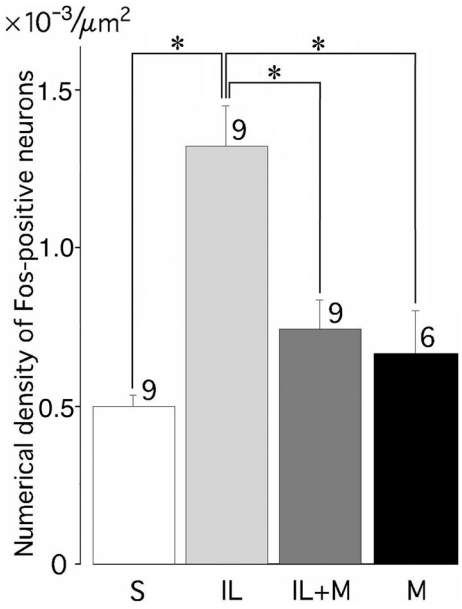

Figure 7 Numerical density of Fos-positive neurons in the total PVN, 90 min after ICV-administration of saline (S), 10 pg/animal IL-1β (IL), 10 pg/animal IL-1β+30 ng/animal α-MSH (IL+M) or 30 ng/animal α-MSH (M). N is indicated on top of each column. Means+S.E.M. * P≤0.001.

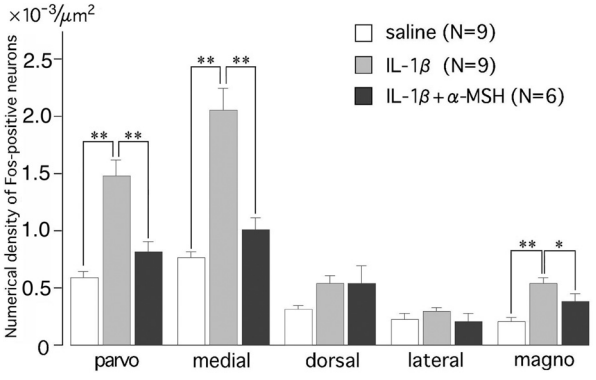

Figure 8 Numerical density of Fos-positive neurons in the PVN and its subregions, viz. the parvocellular area (parvo), medial, dorsal and lateral subregions of the parvo, and the magnocellular area (magno), 90 min after ICV-administration of saline, 10 pg/animal IL-1β or 10 pg/animal IL-1β+30 ng/animal α-MSH. Means+S.E.M, * P≤0.05, ** P≤0.001.

neurons increased in the PVN, especially in the medial part of the parvocellular region. This may mean that the PVN has not only a role in hyperalgesia but also in a second process. In view of the involvement of the PVN in the

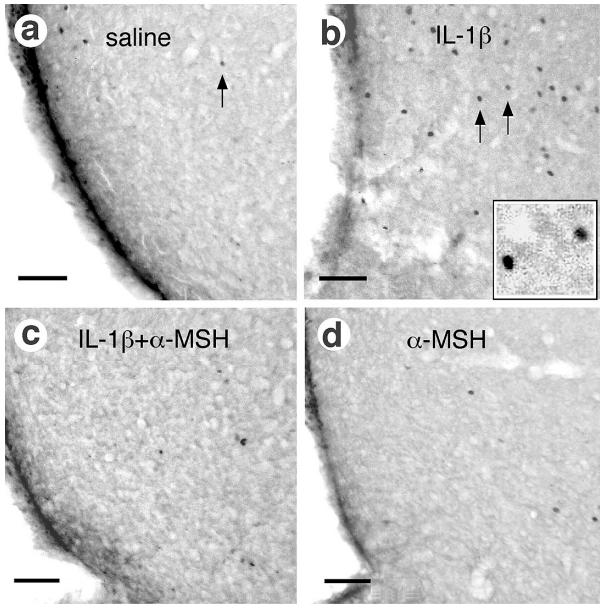

Figure 9 Fos-positive neuronal nuclei (arrows) in the ARC, 90 min after ICV-administration of saline (a), 10 pg/animal IL-1β (b; IL-1β), 10 pg/animal IL-1β+30 ng/animal α-MSH (c; IL-1β+α-MSH) or 30 ng/animal α-MSH (d; α-MSH). Inset in b shows detail of Fos-positive nuclei. Scale bar =50μm.

control of the hypothalamo-hypophyseal axis, this action may be related to a prolonged stress-adaption response. Co-injection of α-MSH with a hyperalgesic dose of IL-1β prevents induction of Fos-expression in the PVN. This result demonstrates that α-MSH can antagonize IL-1β.

Fos expression in the ARC (Figure 9, 10)

The question arises as to the source of this α-MSH. Therefore we studied the ARC and the PI, both containing α-MSH-producing cells. Following ICV administration of a hyperalgesic dose of IL-1β, Fos-induction was observed in the ARC but not in the PI, whereas the Fos-induction in the ARC was completely abolished by co-injected α-MSH. This fact suggests that the ARC rather than the PI is involved in antagonizing the hyperalgesic effect of IL-1β.

Conclusion

This study provides evidence that ICV

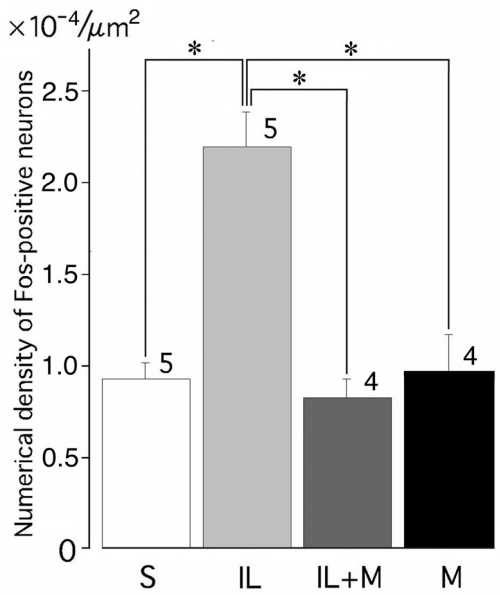

Figure 10 Numerical density of Fos-positive neurons in the ARC, 90 min after ICV-administration of saline (S), 10 pg/animal IL-1β (IL), 10 pg/animal IL-1β+ 30 ng/animal α-MSH (IL+M) or 30 ng/animal α-MSH (M). N is indicated on top of each column. Means+S.E.M. * P≤0.001.

injection of a hyperalgesic dose of IL-1β activates both the PVN and the ARC, and that α-MSH produced in the ARC, but probably not in other parts of the brain and not in the pituitary gland, antagonizes this effect of IL-1β, possibly by inhibiting PVN activity.

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