Zeitschrift: Bulletin der Schweizerischen Akademie der Medizinischen Wissenschaften = Bulletin de l'Académie suisse des sciences médicales = Bollettino dell' Accademia svizzera delle scienze mediche

Herausgeber: Schweizerische Akademie der Medizinischen Wissenschaften

Band: 34 (1978)

Artikel: Interaction of -melanotropin with central dopamine systems : role of hormonal state and molecular structure

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DOI: https://doi.org/10.5169/seals-308151

Bull. Schweiz. Akad. Med. Wiss. 34, 197-208 (1978)

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INTERACTION OF α-MELANOTROPIN WITH CENTRAL DOPAMINE SYSTEMS: ROLE OF HORMONAL STATE AND MOLECULAR STRUCTURE

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Summary

The tubero-infundibular and nigrostriatal DA neurone systems of rats respond to systemic (i.p.) injection of α-MSH (2-100 µg/kg). The response of the tubero-infundibular (arcuate) DA neurones, an increase in cellular fluorescence intensity which can be interpreted as a sign of increased neuronal activity, is essentially the same in males, estrogen-progesterone-pretreated ovariectomized females and hypophysectomized males, whereas the type of response elicited by α-MSH in the nigral DA neurones depends upon the hormonal state of the animal. Differences between the two DA neurone groups exist also with regard to the effects of peptide fragments containing the two active sites of the α-MSH molecule. Results of lesion experiments in the lower brainstem (area postrema) and of blockade of muscarinic mechanisms by atropine further point to differences in the mechanisms underlying the peptide effects on the two neurone systems. The reaction of the tubero-infundibular DA system (which controls the pars intermedia of the pituitary) can be considered to reflect the activation of a feedback mechanism on MSH secretion, while the functional counterpart of the changes observed in the nigral system remains unknown at the present time.

Zusammenfassung

Tubero-infundibuläre und nigro-striatale dopaminerge Neurone der Ratte reagieren auf intraperitoneale Injektion von α-MSH (2-100 µg/kg). Die Reaktion der tubero-infundibulären dopaminergen Neurone (Nucleus arcuatus) besteht in einem Anstieg der zellulären Fluoreszenzintensität, welcher als ein Zeichen erhöhter Aktivität interpretiert werden kann. Dieser Effekt ist grundsätzlich gleich bei männlichen, Oestrogen-Progesteron-vorbehandelten ovariektomierten weiblichen sowie hypophysektomierten männlichen Ratten. Demgegenüber hängt die Antwort der nigralen dopaminergen Neurone auf α-MSH-Injektion von der

Hormonsituation des Tieres ab. Unterschiede zwischen den beiden Dopaminzellgruppen bestehen auch mit Bezug auf die Wirkung von Peptidfragmenten, welche die zwei aktiven Stellen des α-MSH-Moleküls enthalten. Ergebnisse von Läsionen im unteren Hirnstamm (area postrema) und Blockade der muscarinischen Rezeptoren durch Atropin ergeben weitere Hinweise auf Unterschiede zwischen den Peptideffekten auf die zwei Neuronensysteme. Die Antwort des tubero-infundibulären Dopaminsystems, welches die Sekretion des Hypophysen-Zwischenlappens reguliert, entspricht wahrscheinlich einer Feedback-Kontrolle der MSH-Sekretion, während die Bedeutung der Reaktion des nigralen Dopaminsystems vorläufig ungeklärt bleibt.

The pars intermedia of the hypophysis is well developed in many mammals, also in rat and mouse, but its role remains somewhat enigmatic in this class of animals. Among the most interesting and also well documented actions of pars intermedia hormones such as α-melanotropin (α-MSH) and related peptides in mammals are effects on the central nervous system, i.a. on behaviour (DE WIED, 1969; KASTIN et al., 1973). Moreover, another type of centrally active peptides, endorphins, have recently been localized in pars intermedia cells (BLOOM et al., 1977). There are thus two interesting aspects linked with the search for interactions of central neurotransmitter systems with the intermediate lobe axis, i.e., their involvement in the control of pars intermedia function as well as their participation in processes related to effects of peptides on other brain functions.

1. Effects of α-MSH on central dopamine systems and hormonal state

The control of pars intermedia function is exerted to an important part (possibly besides other mechanisms) by the tubero-infundibular dopamine (DA) neurone system which in mammals innervates this part of the pituitary directly (BJÖRKLUND et al., 1973). Catecholamines have been found to inhibit the secretion of MSH, most probably by a direct action on the glandular cells (TALEISNIK et al., 1972; BOWER et al., 1974; SMITH, 1975; ITO, 1974; TILDERS and MULDER, 1975; BAKER, 1976; MORGAN and HADLEY, 1976). In spite of the differences in the anatomical arrangement, the situation thus bears some similarities with that observed with prolactin (cf. MACLEOD, 1976). One interesting aspect of the participation of the DA neurones in the regulation of MSH secretion can be seen in the fact that these neurones are influenced from various brain regions that are engaged in the integration of hormonal and behavioural effects (LICHTENSTEIGER and KELLER, 1974; LICHTENSTEIGER and LIENHART, 1975a). Some of these influences may be related to the control

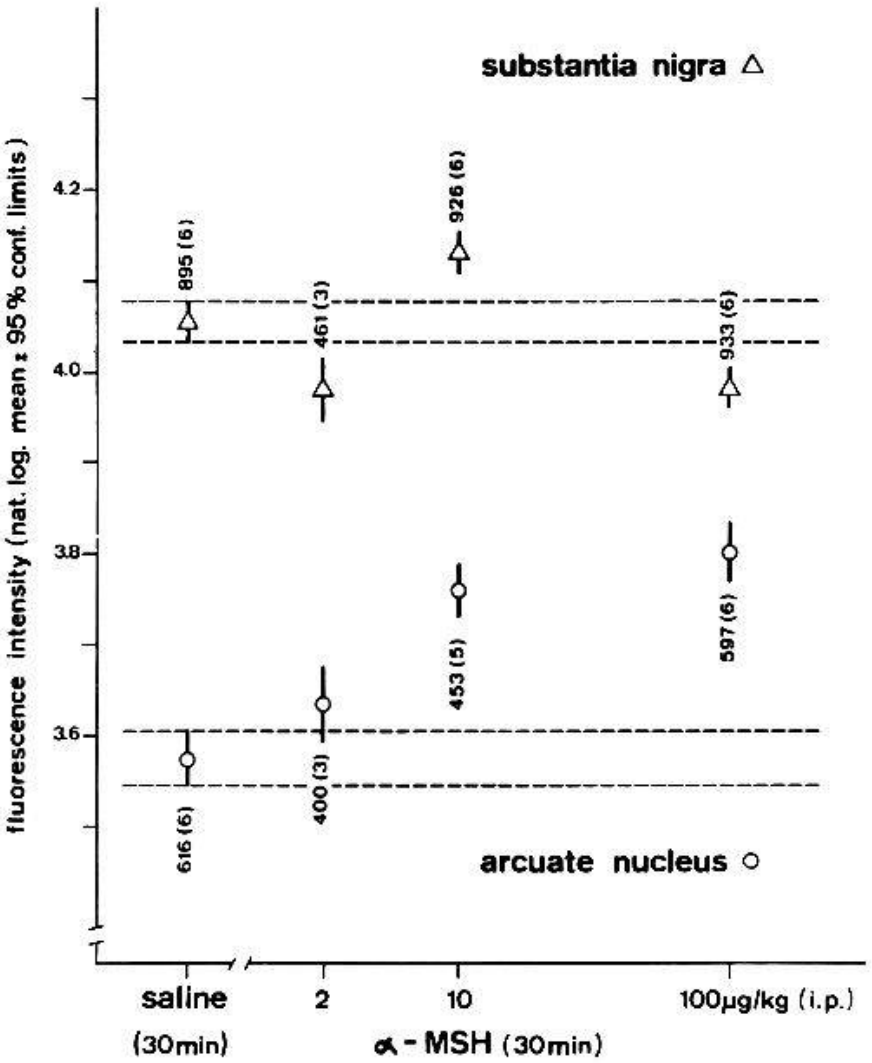

Fig. 1. Response of DA neurones in the arcuate nucleus of the hypothalamus and in substantia nigra of male rats to i.p. injection of α-melanotropin (α-MSH). Mean fluorescence intensities of experimental groups calculated from logarithmically transformed intensity values of individual cells (natural logarithm of 100 x i_r; i_r = (absolute intensity of a cell minus mean tissue background of the section) / (mean intensity of noradrenaline-containing standard minus mean intensity of noradrenaline-free standard of the same section), cf. LICHTENSTEIGER et al., 1976), with 95 % confidence limits, cell count and, in brackets, number of rats. The control levels of the two neurone groups are typically observed at different levels, as a rule slightly above 4.0 in substantia nigra and around 3.6 in the arcuate nucleus.

of the centrally active hormones of pars intermedia. It seems noteworthy, e.g., that the tubero-infundibular DA neurones are influenced by electrical stimulation in posteromedial thalamus or dorsal hippocampus (LICHTENSTEIGER and LIENHART, 1975a) which are important for behavioural actions of α-MSH (BOHUS and DE WIED, 1967), but one should not overlook the fact that the secretion of anterior lobe hormones is also affected from those regions (VELASCO and TALEISNIK, 1969; GALLO et al., 1971; CÁCERES and TALEISNIK, 1977).
In view of their functional position, the tubero-infundibular (arcuate) DA neurones, or part of them, could be expected to react to changes in blood levels of α-MSH. In addition, we

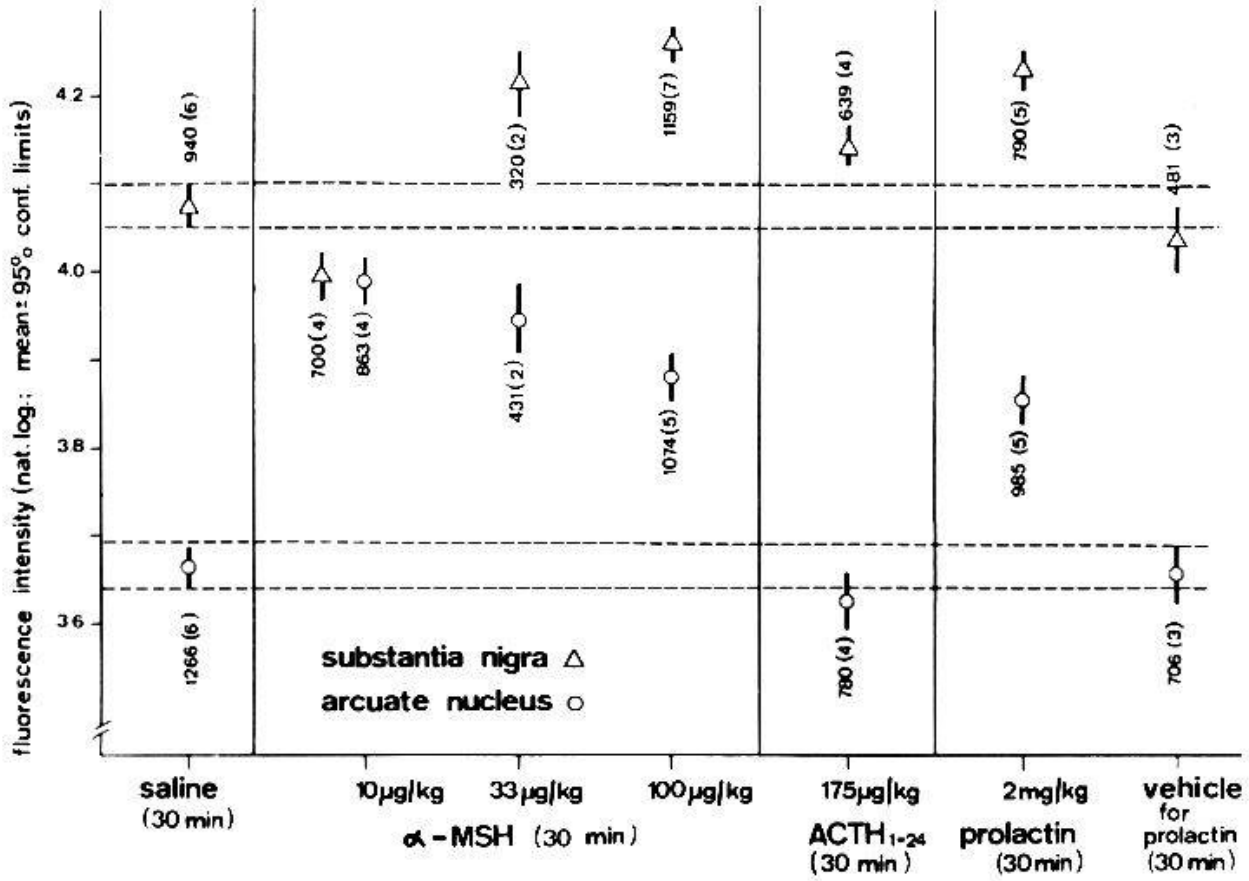

Fig. 2. Effect of α-MSH, ACTH 1-24 and prolactin on DA neurones in arcuate nucleus and substantia nigra of female rats ovariectomized for 3 weeks and pretreated for 1 day with 5 µg estradiol-dipropionate and 2 mg progesterone (s.c.). Mean fluorescence intensities of experimental groups as in Fig. 1 (calculated from data in LICHTENSTEIGER and LIENHART, 1977). Both DA neurone groups were influenced by α-MSH and prolactin (in a dose approximately equimolar to 100 µg/kg α-MSH), whereas ACTH 1-24 remained without effect on the arcuate DA neurones and had no certain effect on the nigral system.

have studied the nigral DA neurones in the same animals as an example for an extrahypothalamic DA system. It was chosen i.a. because we had observed that stimulatory effects from several extrahypothalamic areas reached the nigral as well as the tubero-infundibular DA neurone group (LICHTENSTEIGER and LIENHART, 1975a). The functional state of the two neurone groups was assessed by histochemical microfluorimetry; according to correlative electrophysiological-micro-fluorimetric studies on nigral DA neurones, cellular fluorescence intensity of DA neurones reflects their firing rate (LICHTENSTEIGER et al., 1976: Increase in firing rate accompanied by increased intensity).

Investigations on male rats (Fig. 1) and on females that had been ovariectomized and pretreated for 1 day with estrogen and progesterone (Fig. 2, LICHTENSTEIGER and LIENHART, 1975a, 1977), revealed that both DA neurone groups responded to systemic (i.p.) injection

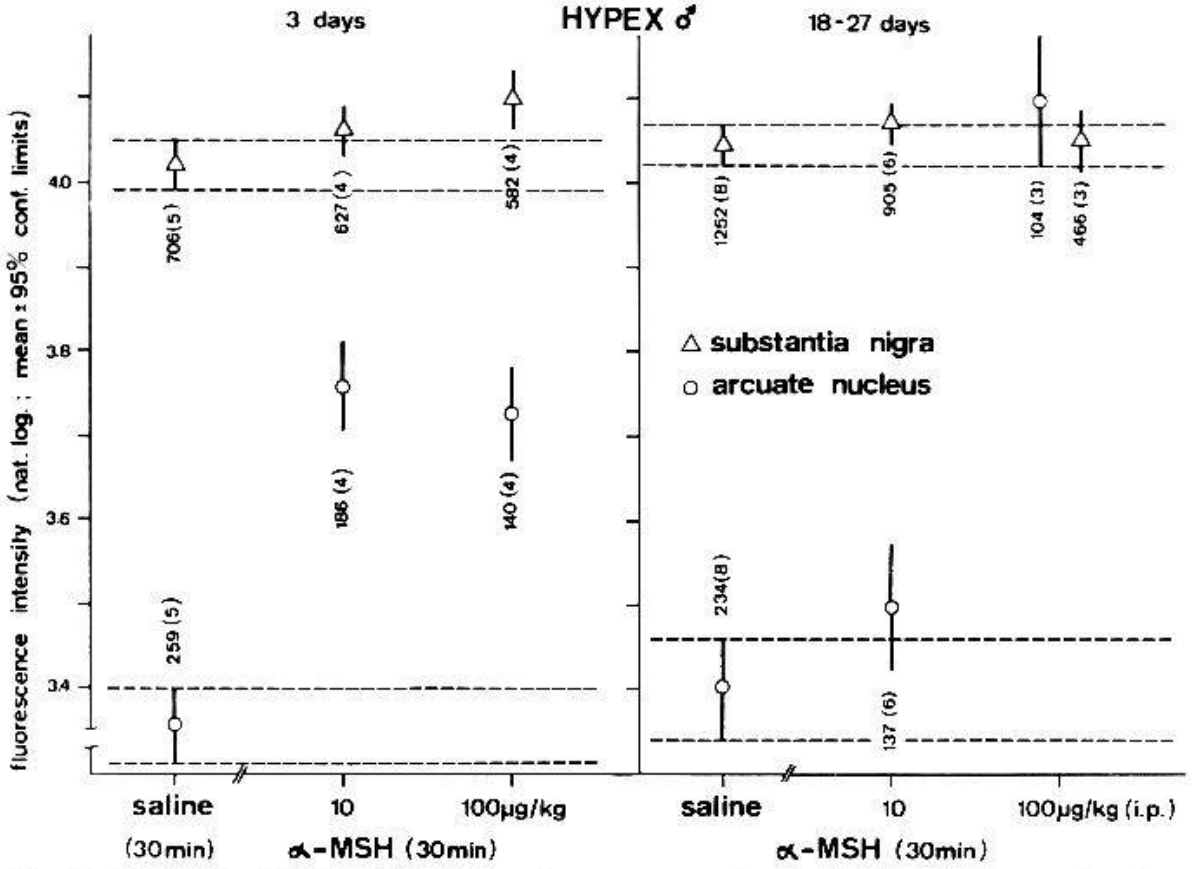

Fig. 3. Effect of α-MSH on DA neurones in arcuate nucleus and substantia nigra of male rats after hypophysectomy. Mean fluorescence intensities of experimental groups as in Fig. 1.

of α-MSH. The arcuate DA neurones consistently exhibited an increase in cellular fluorescence intensity. On the basis of the correlative studies mentioned above, this intensity change can be interpreted as reflecting a rise on MSH secretion. The nigral DA neurones were also influenced, yet, in contrast to the arcuate group, their response depended upon the hormonal state of the animal. In the females, a simple dose-response relation was observed, with a type of response similar to that of the arcuate DA neurones. In the males, a slight but significant rise in cellular fluorescence intensity was seen after a lower dose that had been ineffective in females, whereas the higher dose tended to depress intensity to below control level.

In order to further elucidate the possibility of an influence from other pituitary axes on the expression of the peptide effect, we repeated the experiments on hypophysectomized male rats (Fig. 3). Experiments were performed on acutely and chronically hypophysectomized rats. The results obtained with the former group appear to be more reliable especially as far as the arcuate DA neurones are concerned, since we observed a marked reduction in the number of visibly fluorescent catecholamine neurones in the arcuate nucleus 3-4 weeks after hypophysectomy. Also in the acutely hypophysectomized rats, the reduced mean fluo-

rescence intensity of the arcuate DA neurones of the controls indicated a change in the functional state of this neurone group. The reaction to α-MSH persisted in the tubero-infundibular DA neurones. In contrast, the response of the nigral DA neurones to the peptide was again altered: The effect of the 10 µg/kg dose disappeared and instead, intensity tended to be elevated after the highest dose.

It appears from these results that the activation of the tubero-infundibular DA neurones by systemic administration of α-MSH results from an action of this peptide itself. It seems conceivable that this activation was brought about by mechanisms which are operating in a physiological feedback system, although at considerably lower plasma concentration of the peptide. We have not yet analyzed our data separately for the 10 antero-posterior levels studied in the arcuate nucleus, but it has become evident that a response can also be observed in the posterior part of this cell group, although according to BJÖRKLUND and his coworkers (1973), only the anterior part projects to the intermediate lobe. Whether the general reaction of the arcuate DA group represents a non-physiological situation resulting from the marked elevation of plasma levels of α-MSH, or whether crossed feedback effects on other pituitary axes are mediated by the tubero-infundibular DA neurones also under physiological conditions, cannot be decided at the present time. In the females, α-MSH injections tended to reduce prolactin levels (LICHTENSTEIGER et al., 1977). The variations observed in the reaction of the nigral DA neurone group contrast with the consistency of the arcuate response and suggest that in the case of the nigrostriatal system, a pituitary factor or a factor depending upon the pituitary, influences the action of α-MSH. It should be noted that other types of changes of the functional state of the central nervous system also appear to modify the effect of α-MSH on the nigral DA system: In two preliminary experiments on ovariectomized, steroid-pretreated rats, we observed that α-MSH administered in urethane anesthesia depressed firing of neurones in zona compacta of substantia nigra and reduced the fluorescence intensity of the DA neurones (FELIX and LICHTENSTEIGER, unpublished observations).

2. Role of the active sites of α-MSH in its effect on DA neurones

α-MSH has been found to possess two active sites, an N-terminal one in the region 4-10, i.e., the region of the active site of ACTH (with some differences in the relative importance of individual amino acids), and another one at the C-terminal (11-13; EBERLE and SCHWYZER, 1976). We wondered which site(s) might be responsible for the complex pattern of α-MSH actions on the two DA systems. In male rats, the arcuate DA neurones did not respond at all to the administration of ACTH 4-10 in a dose range comparable to that of α-MSH

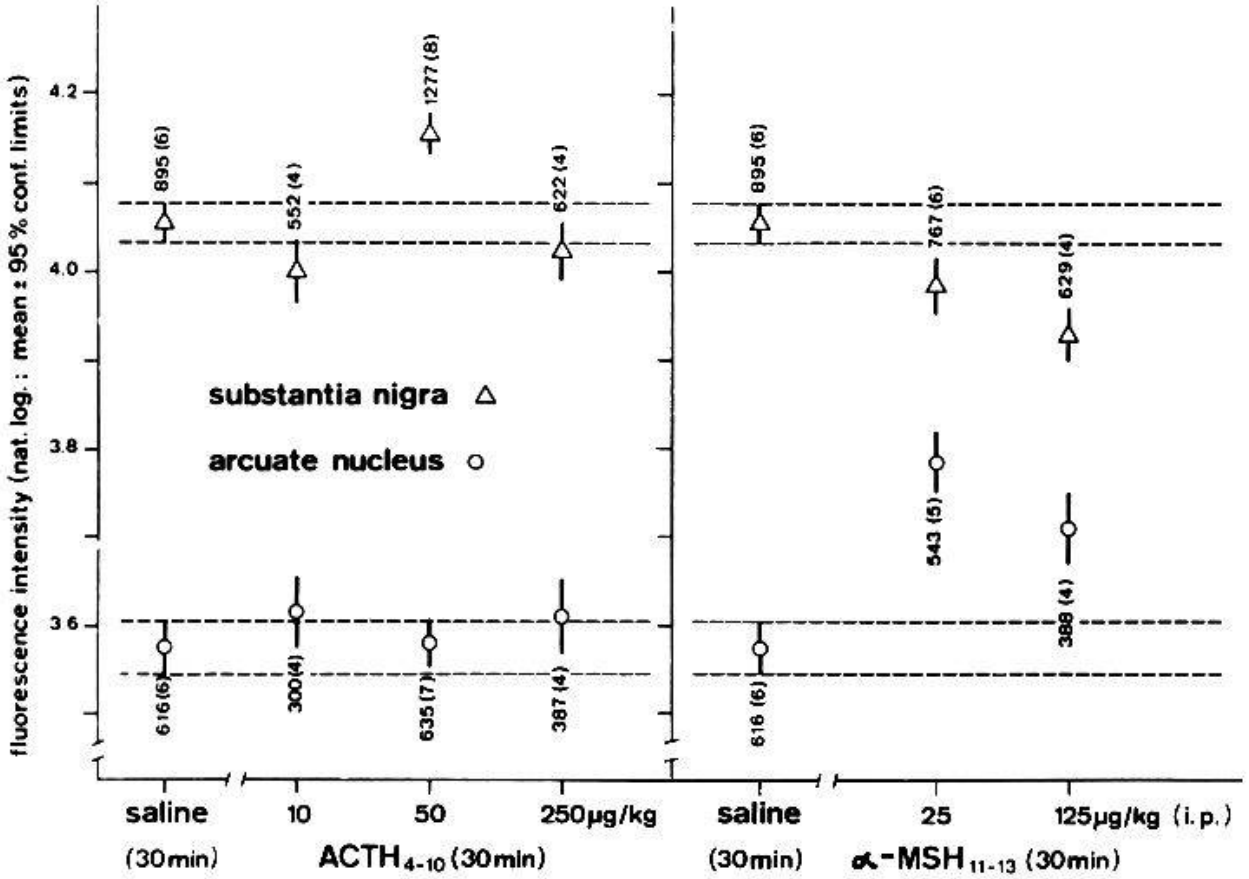

Fig. 4. Reaction of DA neurones in arcuate nucleus and substantia nigra of male rats to peptide fragments containing the two active sites of α-MSH (ACTH 4-10 = sequence 4-10 without N-acetylation, α-MSH 11-13 = sequence 11-13 with N-acetylation). Mean values of experimental groups as in Fig. 1.

and which has been found to be centrally active (Fig. 4). In contrast, the N-acetylated sequence 11-13 (α-MSH 11-13) elicited a rise in fluorescence intensity as did α-MSH. Both fragments appeared to influence the functional state of the nigral DA system, but in different ways: ACTH 4-10 elicited a slight increase in fluorescence intensity at an intermediate dose, i.e., produced a picture similar to that after α-MSH in the male, whereas after α-MSH 11-13, a dose-related, marked decrease in fluorescence intensity was observed (indicating inhibition of the neurones).

These results further indicate that the mechanisms underlying the response of the two DA systems to α-MSH probably differ, in spite of parallel changes under certain conditions. The observations on the arcuate DA neurones support the idea that their activation by α-MSH occurs in the context of feedback regulation, since the C-terminal active site appears to be specific for α-MSH. The lack of an effect of ACTH 4-10 is in keeping with our earlier observation on ACTH 1-24 in the females (Fig. 2) and agrees with certain data suggesting that the tubero-infundibular DA system is less important in ACTH control (SCAPAGNINI et al.,

1972). It should be noted that in a preliminary experiment in female rats, ACTH 4-10 was found to influence the arcuate DA neurones (LICHTENSTEIGER et al., 1977), which again points to the role of the hormonal state and of possible interactions with other factors.
On the basis of our present data, it cannot be decided whether the differential action of the two peptide fragments on the nigral DA neurones really reflects the existence of two different mechanisms, or whether both types of changes in the nigral DA neurones (rise and decrease in intensity) might be encountered with both fragments if the dose ranges were further extended or the functional state of the animal changed. In any case, the sequence 4-10 appears to exert certain effects on the nigral DA system, in contrast to the tubero-infundibular DA neurones of the male. This opens up the possibility of interactions with ACTH and related peptides and with peptides and other substances that interact themselves with ACTH, e.g., at opiate receptors (TERENIUS, 1976; GISPEN et al., 1976). We have previously noted that α-MSH was capable of antagonizing the rise in fluorescence intensity induced by morphine in nigral DA neurones of male mice and delayed the onset of increased locomotor activity by about 15 min (LICHTENSTEIGER and LIENHART, 1975b).
In studies on male rats anesthetized by urethane, mean relative fluorescence intensity of nigral DA neurones has been found to be correlated with mean firing rate (LICHTENSTEIGER et al., 1976). A tentative estimation of the changes in mean firing rate of the nigral DA neurones brought about in males by the various peptides can be made on the basis of this correlation, provided the factors determining this correlation were essentially the same in the two series of experiments (i.e., the regression function between firing rate and cellular intensity were the same quantitatively as in the previous experiment). A definite answer to this latter question cannot yet be given, since the biochemical background of the cellular intensity changes has not been fully elucidated. In figure 5, we have tried to illustrate the relationship between intensity and firing rate, using the regression based on a lognormal distribution of intensity values and a Poisson-type distribution of firing rates which gave the best fit in the previous study (LICHTENSTEIGER et al., 1976). It appears from this figure that the peptides elicited changes, in the males, in the order of 30 - 40 % of the mean firing rate of the controls. Although the absolute changes are comparatively small, they are nevertheless considerable for the nigral DA neurone group which is characterized by a relatively slow and very regular firing rate. For comparison, it may be mentioned that nicotine elicited an increase from 5,3/sec to 7,6/sec (0,33 mg/kg s.c.) or 11,4/sec (1 mg/kg) in urethane-anesthetized males. The use of the correlation for quantitative estimates in female rates is linked with more uncertainties because it cannot be excluded a priori that the biochemical

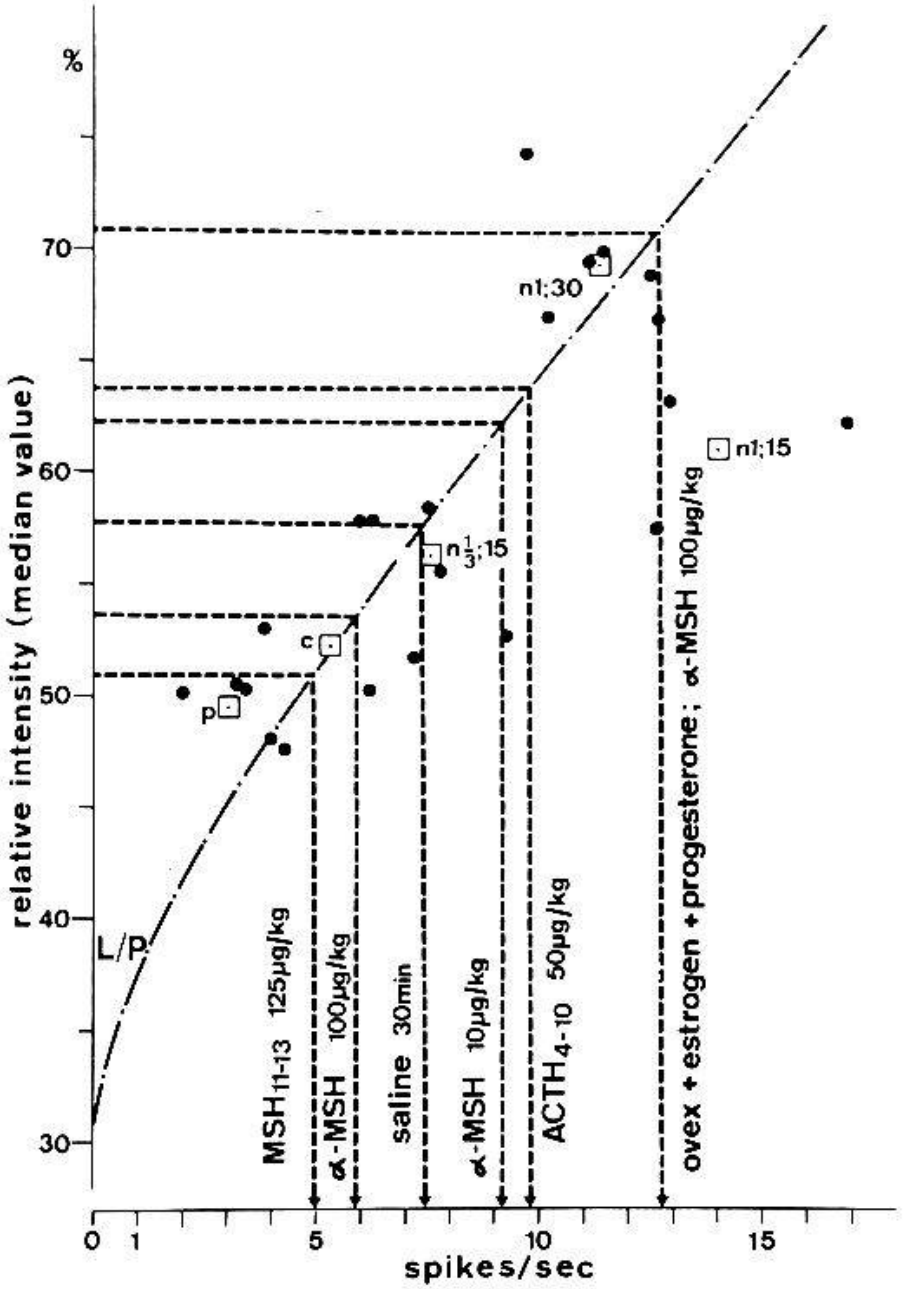

Fig. 5. Relation of cellular fluorescence intensity of nigral DA neurones and firing rate (based on data from LICHTENSTEIGER et al., 1976).

process underlying the histochemical intensity changes may be influenced by the hormonal state, i.e., that the change in intensity per change in firing rate may not be exactly the same in male and female rats.

3. Conclusions

Systemic administration of α-MSH in doses that are equal to or below those usually used in behavioural studies affects the functional state of both DA neurone groups. Certain differences have become apparent, however, upon closer examination of the peptide effects: In the

dose range studied, the tubero-infundibular DA neurones exhibited a very constant type of response with only certain variations in its magnitude. This response appears to be linked mainly with the C-terminal active site of the α-MSH molecule. Although very high plasma levels of α-MSH were obtained with the doses used (KOPP and LICHTENSTEIGER, unpublished observations), it seems conceivable that the injection revealed a feedback loop on MSH secretion that is operating also under physiological conditions. The reaction of the nigral DA neurones appears to be based on different mechanisms, as it can be either stimulatory or inhibitory depending upon other factors, and is linked with both active sites of α-MSH. This differential responsiveness of the two DA neurone groups is further reflected by differences in the role played by certain brain structures and neurotransmitter systems in the manifestation of the peptide effect: In female rats, lesions of area postrema blocked the effect of α-MSH on the arcuate DA neurones and changed the nigral response from a rise into a decrease in cellular fluorescence intensity. Thus ascending influences appear to be of importance for the reaction of both DA neurone groups, but some additional factors appear to interact with the nigral reaction (LICHTENSTEIGER and LIENHART, 1977). Moreover, results obtained with atropine indicate that muscarinic mechanisms are essential for the stimulatory nigral response in females, whereas no significant effect was seen with respect to the arcuate DA neurones.

Acknowledgements

This study was supported by SNSF grant 3.669-0.75 and the Hartmann-Müller Stiftung. We thank Dr. H.M. Greven, Organon, Oss, The Netherlands, for a gift of ACTH 4-10 and Dr. A. Eberle, Institut für Molekularbiologie, Eidgenössische Technische Hochschule, Zürich, for α-MSH and α-MSH 11-13.

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