INFLUENCE OF ACUTE AND CHRONIC MORPHINE OR STADOL ON THE SECRETION OF ADRENOCORTICOTROPHIN AND ITS HYPOTHALAMIC RELEASING HORMONE IN THE RAT

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SUMMARY: The effects of acute and chronic treatment with morphine and stadol on the functional activity of the hypothalamo-pituitary-adrenocortical (HPA) system in the rat were studied by investigating their effects on the secretion of adrenocorticotrophin (ACTH) by the pituitary gland and corticotrophin-releasing hormone (CRH) by the hypothalamus. Acute injection of morphine or stadol (3.5 mg/100 g body weight i. p.) caused a rise at 5 and 25 min followed by a fall at 90 and 120 min in the concentrations of ACTH in the plasma and adenohypophysis and in hypothalamic CRH content. It appears that, in the rat, the response of HPA system to acute morphine or stadol administration could change depending upon the time of courses. In addition chronic morphine or stadol administration (0.5 mg/100 g body weight i. p. daily) for a period of 7 days have little effect on plasma and adenohypophysis ACTH concentration and hypothalamic CRH content. This may indicate that drug tolerance might have developed. Conversely, repeated daily doses of morphine or stadol (2 mg/100 g body weight i. p.) for 7 days cause a significant lowering of plasma and pituitary ACTH concentrations and hypothalamic CRH content. These data suggest that the affect of both drugs is dose related. Overall, the present results are consistent with an increased release of pro-opiomelanocortin-derived peptides after acute morphine or stadol treatment for a short-term, and with a decreased release of these peptides in chronic treatment. However, the results indicate that morphine and stadol change HPA activity by acting on specific receptors in the hypothalamus and raise the possibility that opioid peptides and their receptors in the physiologically important in the control of the secretion of CRH.

Key Words: Morphine, stadol, ACTH, CRH

INTRODUCTION

The rapidity with which the pituitary can modify adrenocortiocotrophic hormone (ACTH) in response to opioid substances (1-4) suggests that the nervous system must play a role in regulating pituitary function. The hypothalamus is particularly implicated in this regulatory process (3,5). Stimulation of selected hypothalamic receptors (6) has been shown to provoke increased secretion of ACTH (3,4) while lesions in specific areas of the hypothalamus prevent the usual response of the anterior pituitary (4). Hypothalamic influence is considered to be mediated by release of a neurohormone from the median eminence into the portal blood which carries it to the adenohypophysis.

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Hypothalamo-pituitary-adrenocortical (HPA) activity in the rat is influenced markedly by opioid substances (3,4,7-9) which appear to stimulate the secretion of corticotrophin releasing hormone (CRH) by acting on specific naloxone-sensitive opioid receptors in the hypothalamus (3,4,6,7).

The acute administration of morphine or related drugs causes a close-related stimulation of the HPA axis (3,4,9). Chronic opiate treatment characteristically results in tolerance to this effect (2,4). Furthermore, Bertolini *et. al.* (10) demonstrated that hypophyseal peptides can modify the development of physical dependence and tolerance to morphine. On the other hand, studies have shown that chronically administered opiates affect pituitary proopiocortin synthesis

and/or secretion (11-13), but the findings are inconsistent. However, the effects of chronic opiate administration upon HPA activity are less well understood.

To our knowledge, the effects of morphine and stadol on plasma and pituitary ACTH and hypothalamic CRH in rat are less well understood. Our previous studies (9) have demonstrated that acute and prolonged administration of morphine to rats could play an important role in the regulation of pituitary-adrenocortical function. In the present work we extended the investigation to study the effects of acute and chronic morphine or stadol treatment in rats on the hypothalamo-pituitary-adrenocortical activity as determined by evaluating their effects on plasma and pituitary ACTH concentrations and hypothalamic CRH content.

MATERIALS AND METHODS

Animals: Adult male albino rats Rattus norvegicus, weighing 120-140 g, were housed 5 per cage and kept for at least 1 week in a room with controlled lighting (lights on 07.00-19.00 h) in which the temperature was maintained at 21-23°C. The animals were handled regularly and food and water available ad libitum. They were always killed between 08.00 and 10.00 h in order to avoid any changes associated with the circadian rhythm.

Drugs: The drugs used during the experiments of this study were morphine sulfate and stadol. Morphine sulfate was obtained from Egypt Company for Medical Preparation, El-Mataria, Cairo, Egypt; while stadol (butorphanol taratrate) was obtained from Bristol Laboratories (Division of Bristol-Myers Company Syracuse, New York, USA). The two drugs were diluted by 0.9% saline solution and were injected intraperitoneally (i. p.).

Drug treatment

Acute treatment: On the experimental days, the procedure was as follows: Experiment 1 (treated animals with morphine): Four different groups, each group includes 5 rats, were given i. p. morphine (3.5 mg/100 g body weight in a volume of 0.5 ml/100 g body weight) and were killed at 5, 25, 90 and 120 min after injection. Experiment 2 (treated animals with stadol): Another four different groups, each of which includes 5 rats, were treated by stadol (3.5 mg/100 g body weight in a volume of 0.5 ml/100 g body weight) in the same way as the morphinized group. Experiment 3 (control animals): Another four different groups, each includes 5 rats, were given i. p. physiological saline (0.9% NaCl). Animals in experiments 2 and 3 were killed at the same time schedule for morphinized treated rats.

Chronic treatment: Five groups of animals each of 5 rats were used in the chronic experiments. Groups 1 and 2 were injected i. p. with morphine in doses of either 0.5 or 2 mg/100 g body weight in a volume of 0.5 ml/100 g body weight daily for 7 consecutive days. Rats in groups 3 and 4 were treated by stadol in the same way as the morphine treated groups. Animals in group 5 were injected with physiological saline for 7 consecutive days. All animals of chronic five groups were killed 30 min later after the last injection.

Collection of blood: The rats were killed by decapitation. Blood was collected from the trunks into chilled plastic heparinized tubes and centrifuged immediately at 2500 rpm and 4°C for 10 min. The plasma was then transferred to new tubes and stored at -30°C for later assay of ACTH.

Extraction of anterior pituitaries and hypothalamus: The anterior pituitary and hypothalamus were removed from rats immediately after decapitation. Each anterior pituitary was homogenized in 2.0 ml 0.1 N HCl and kept at 4°C for 24 h, an aliquot of the supernatant was assayed for ACTH; another aliquot was used for determination of protein. Each hypothalamus was ground in 10 μl 0.1 MHCl and stored on ice for 1 h 1.0 ml artificial cerebrospinal fluid was then added, the mixture shaken thoroughly and centrifuged at 2500 rpm for 5 min. The supernatant fluid was kept on ice for not longer than 1 h before its CRH content was determined.

Methods: ACTH concentrations in plasma and pituitary was determined by an ACTH RIA technique. RIA kit was provided by Diagnostic Systems Inc. 445 Medical Center Blvd., Webster, Texas 77598, USA. Protein of anterior pituitary was measured according to the method of (Bradford (14)).

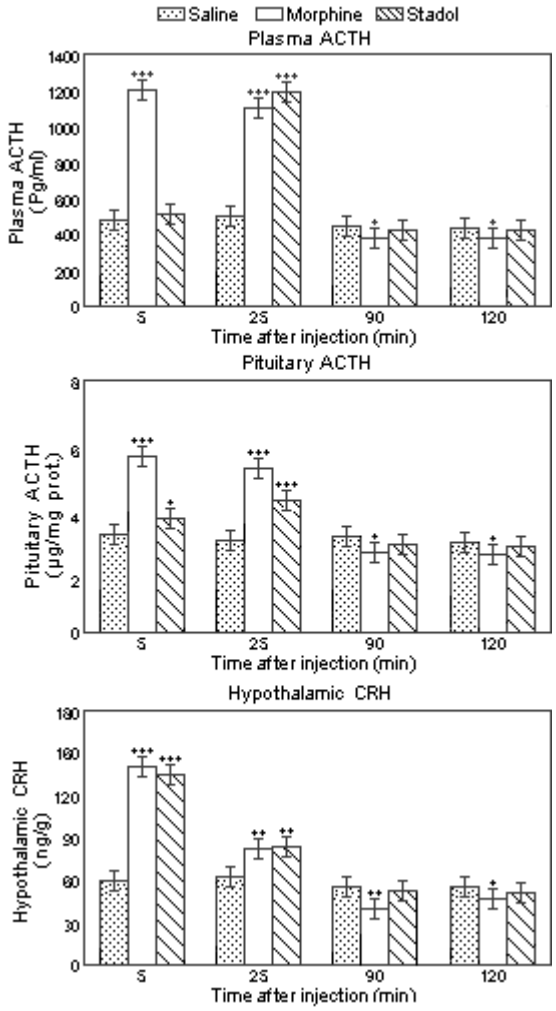
CRH was measured by a RIA. Using synthetic rat/human CRH-41 as standard and rat/human CRH antiserum raised in rabbit rC70 in a final dilution of 1:400.000. The 125 I-labeled rat/human CRH was purchased from New England Nuclear Research Products-DuPont (Boston, MA). Separation of the antigen-antibody complex was achieved by means of a second antibody, goat antiserum to rabbit immunoglobulin G, and 15% polyethylene glycol procedure. The intra- and inter-assay coefficients of variation were less than 13%.

Statistics: The results are reported as the mean ± SE. Differences between means were determined by using ANOVA and Student's *t*-test for individual comparison. Changes were considered significant when the p value was less than 0.05.

RESULTS

Effect of acute morphine and stadol treatment on plasma and pituitary ACTH concentrations and hypothalamic CRH content: As shown in Figure 1, the data of ACTH level in plasma reflect the occurrence of a significant ($p<0.001$) increases in its level at 5 and 25 min after acute morphine administration and at 25 min of acute stadol administration as compared to the saline groups. On the other hand, there was a significant ($p<0.05$) decrease in plasma ACTH level at 90 and 120 min after morphine injection compared to ACTH level in the control saline animals. However, non-significant ($p>0.05$) decreases were recorded in

Figure 1: Plasma ACTH level, Adenohypophyseal ACTH concentration and Hypothalamic CRH content at various times in rats acutely injected i.p. with saline or morphine (3.5 mg/100 g body weight i.p.) or stadol (3.5 mg/100 g body weight i.p.). Each bar is the mean ± SE of 5 animals *p<0.05, ***p<0.001 vs. the saline group.



ACTH levels after 90 and 120 min of stadol treatment exhibiting a level almost matching that of the control saline groups.

Pituitary ACTH content is shown in Figure 1. ACTH concentrations in adenohypophysis were significantly increased (p<0.001) at 5 and 25 min after acute morphine administration and at 25 min of acute stadol administration as compared to the saline groups. However, there was a significant (p<0.05) increase in pituitary ACTH content after acute stadol administration in rats at 5 min compared to the saline group. On the other hand, there was a significant decrease (p<0.05) in pituitary ACTH concentration at 90 and 120 min after morphine injection compared to ACTH in the control saline animals. However, non-significant decreases (p>0.05) were record in ACTH content after 90 and 120 min of acute stadol administration.

CRH contents of hypothalamus exhibited significant increases (p<0.001 and p<0.01, respectively) after 5 and 25 min of treatment with either of both drugs. In contrast, the data showed significant decreases (p<0.01 and p<0.05, respectively) in plasma CRH level at 90 and 120 min after morphine administration. However, after 90 and 120 min of treatment with stadol, a limited non-significant (p>0.05) declines were recorded in hypothalamic CRH contents (Figure 1).

Effect of chronic morphine and stadol treatment on plasma and pituitary ACTH concentrations and hypothalamic CRH content: Plasma ACTH levels in rats treated with morphine or stadol (0.5 mg/100 g body weight) for 7 consecutive days showed no significant changes when compared to the saline treated control animals. On the other hand, the data showed a significant (p<0.001) decrease in plasma ACTH level after morphine (2 mg/100 g body weight) administration in rats for one week, whereas there was a significant (p<0.01) decrease in ACTH levels after stadol (2 mg/100 g body weight) injection in rats for 7 consecutive days compared to the saline group (Figure 2).

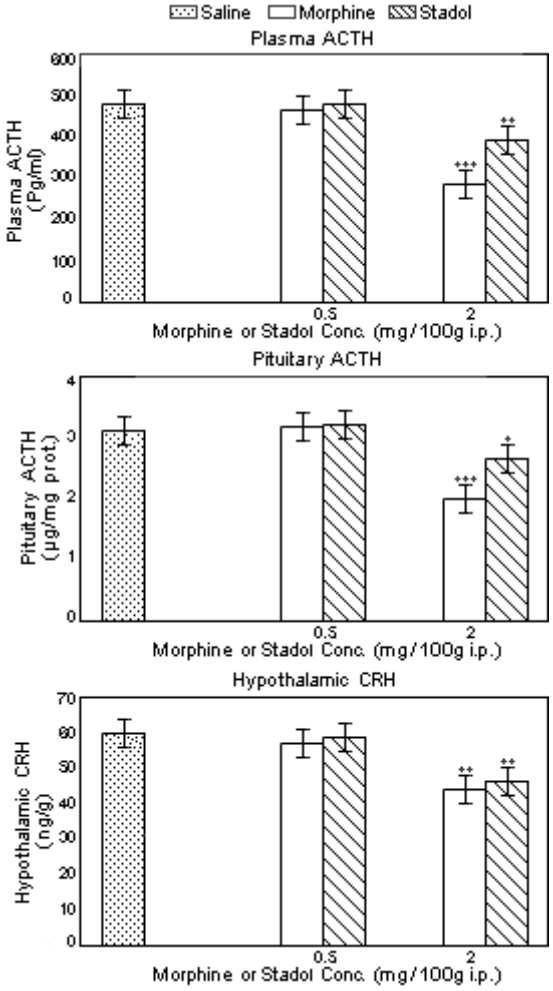
Figure 2 shows no changes in adenohypophyseal ACTH concentrations after chronic morphine or stadol (0.5 mg/100 g body weight) administration for 7 consecutive days when compared to the control saline animals. On the other hand, pituitary ACTH content were significantly (p<0.001 and p<0.05, respectively) lower after morphine or stadol (2 mg/100 g body weight) administration to rats for one week than the corresponding saline rats.

The data record in Figure 2 indicates that no significant changes in hypothalamic CRH contents were observed after 7 consecutive days of morphine or stadol (0.5 mg/100 g body weight) administration, while a significant decreases (p<0.01) after 7 consecutive days of treatment with either of the two drugs (2 mg/100 g body weight) were recorded.

DISCUSSION

Although the literature contains many references to the effect of morphine and opioid peptides on the HPA axis (15-18), few direct estimates of the effect of mor-

Figure 2: Plasma ACTH level, Adenohypophyseal ACTH concentration and Hypothalamic CRH content in rats treated with saline (i.p.) and in rats treated with morphine or stadol (0.5 mg/100 g or 2 mg/100 g body weight i.p.) for 7 consecutive days. Each bar is the mean ± SE of 5 animals *p<0.05, ***p<0.001 vs. the saline group.



phine or stadol on plasma and pituitary ACTH and hypothalamic CRH have been made before. The present data indicate that acute morphine or stadol administration causes an initial hyper-secretion of corticotrophin accompanied by marked elevation in the contents of ACTH and CRH in the adenohypophysis and hypothalamus respectively, especially 5 and 25 min after application of either drugs. Similar stimulatory effects of morphine and opioid peptides on the HPA activity has been observed in several species including the rat (3,4,9,19,20), the mouse (21), the cat (22) and the guinea pig (23,24), whereas in humans they suppress this activity (25). The mechanism whereby morphine stimulates the HPA activity in the rat has been subject of controversy. The acute administration of morphine or related drugs cause a dose-related stimulation of the HPA axis. The doses used in this and other studies (1,3,4,9) are considerably higher than those employed in comparable to humans studies and hence it is possible that the observed endocrinological response is merely due to non-specific 'stressful' actions (e.g. cardiovascular changes) of the drug. Indeed, the increases in plasma and adenohypophysis ACTH concentrations and hypothalamic CRH content, in this study, are similar to those which occur in response to morphine (3,4,6-8), or severe stress (3,26).

Plasma levels of ACTH (a marker of corticotrophin cells) and β-endorphin (β-END) increase after acute injection of morphine (3,4,9,27). Dexamethasone blocks the morphine-induced release of ACTH and β-END (27). These data indicate that the changes in pituitary function seen following morphine administration appear to result in the release of ACTH and β-END from the anterior lobe into blood as a consequence of an increase in CRH (3,5,6).

Therefore, the initial increase in the plasma and adenohypophysis levels of ACTH, especially after 5 and 25 min of morphine or stadol administration, would be due to an enhanced secretory activity of the hypothalamus evoked by the release of CRH (3,5,6). Thus, morphine and stadol would appear to interfere with central nervous system pathways concerned with control of ACTH release.

Our results, about the initial increase in CRH levels after acute injection of both drugs, are similar to those of Buckingham and others (3-7) who demonstrated that morphine and opioid substances stimulate the release of CRH from the hypothalamus. It has also been proposed that the increase in hypothalamic CRH content in rats treated with morphine or stadol may be related to the action of both drugs on specific receptors in the hypothalamus to stimulate the secretion of CRH (3,4,6-8) and suggest that more than one type of opioid receptor is involved (6). It is possible that they also act at extra hypothalamic sites within the brain. However, the presence in the hypothalamus of met-enkephalin and leu-enkephalin in high concentrations (3,26) suggest that the receptors for opioid peptides are physiologically important in the control of CRH secretion. Additionally, the stimulatory effect of morphine and drugs which mimic its actions on CRH secretion are mediated

by μ and *k*-opioid hypothalamic receptors (6). The mechanism responsible for the rapid alteration in the tissue content of the releasing hormone is not known but it may involve the formation of CRH from a biologically inactive precursor molecule (28).

The parallelism between the morphine or stadol-induced hyper-secretion of ACTH and the changes in hypothalamic CRH activity supports earlier suggestions that the opiate acts on the hypothalamus or other brain areas and not on the pituitary gland (3,6,8,21). Confirmation of an action at the hypothalamic level is provided by the in vitro studies (3,29) which demonstrate not only the ability of morphine to stimulate the secretion of CRH by the hypothalamus but also the failure of opioid substances to influence directly the ACTH activity of the pituitary gland (3,29).

In contrast, the results of the present study demonstrates that acute morphine or stadol injection depresses ACTH and CRH at 90 and 120 minutes. This may indicate that an inhibitory feedback effect of the initial increase in plasma ACTH occurred, as have been suggested in several species including the rat (9,30,31) and the anoestrous ewe (32). Those authors have found a delayed decrease in plasma levels of ACTH after an initial increase in the concentration of this hormone, which was explained by a suppression of CRH (present study). Also the concomitant decrease in hypothalamic CRH content might as well stimulate the depression of ACTH concentration in plasma and pituitary gland. In addition, Nikodijevic and Maickel (33) established that i. p. injection of morphine in rats exhibited a stimulating effect on pituitary ACTH hyper-secretion at doses of 3.75 mg morphine sulphate/kg or greater. A maximal effect within 30 min was observed with a dose of 20 mg/kg and lasts for 4-6 h. The duration of action, as measured by elevated plasma corticosterone levels, is 4-8 h; after 24 h, the levels of plasma corticosterone and adrenal ascorbic acid have returned to normal (33).

Repeated daily dosage with morphine or stadol (0.5 mg/100 g) for one week caused non-significant decline in the contents of ACTH and CRH in the adenohypophysis and hypothalamus respectively which may indicate that drug tolerance might have developed in rats in the present study. Similar effects of morphine have been observed on pituitary-adrenocortical system by Nikodijevic and others (9,33-35). Additionally, morphine in low doses has either no effects or modest suppressive effect on basal glucocorticoid secretion in man (36) and rat (37). In contrast, morphine blunts ACTH secretion in pentobarbital-pretreated rats (38) and in patients given diazepam and nitrous oxide who undergo cardiac surgery (39). These findings suggest that the effect of morphine is dose related. Low doses blunt ACTH release, while higher doses stimulate ACTH, possibly by inducing a stress response, which might fit with the present observation, where a dose of only 0.5 mg/100 g was applied. Moreover, George and Kokka (2) demonstrated that chronic opiate treatment characteristically results in tolerance of the HPA axis activity.

The mechanisms whereby morphine inhibits HPA function are not clear. Alternatively, inhibitory actions of morphine may be unmasked by the development of tolerance to the stimulatory effects of the opiate. Buckingham and Cooper (4) found that hypothalami removed from rats receiving prolonged morphine-treatment appeared to be desensitized. Furthermore, the development of the inhibition of HPA function in vivo closely parallels the development of tolerance to the analgesic actions of the opiate.

Buckingham (3) reported that the marked impairment of the HPA activity in rats rendered tolerant to morphine is in accord with earlier findings based on indirect indices of ACTH secretion (1). The inhibition is associated with the inability of the hypothalamus to secrete CRH which suggests that this effect, like the stimulatory one, is also due primarily to actions of the opiate on the hypothalamus or other parts of the brain and not on the pituitary gland or the adrenal cortex.

On the other hand, morphine or stadol in a larger doses (2 mg/100 g daily for a period of one week) induced a marked decrease in ACTH and CRH concentrations. In agreement with such developments the present data suggests that this effect of morphine or stadol is dose related. The depression in ACTH and CRH levels after both drugs injection are similar to those reported previously in morphinized rats (4,9,40) and in heroin addicts (41).

Since β-END and ACTH have a common precursor (42) and are under common hypothalamic control these results led to the hypothesis that there may be a decreased release and possibly, an altered synthesis of pro-opiomelanocortin-derived peptides from the anterior pituitary of morphine or stadol-treated animals (9,20). The mechanisms whereby morphine or stadol inhibits pituitary function are not clear, although this inhibition seems to be associated with inability of the hypothalamus to secrete CRH under the action of both

drugs (present study). Similar effects of morphine have been observed in rats (4). Moreover, Buckingham and Cooper (4) reported that prolonged treatment with opiates in rats resulted in the development of a pharmacological lesion in the hypothalamus which impairs the pituitary-adrenocortical response to stress. Also the present data are consistent with the hypothesis that constant stimulation of opioid receptors activates feedback mechanisms regulating hypothalamic and/or pituitary opioid peptides (17,43), resulting in a decreased release of ACTH from the anterior pituitary gland.

Interestingly, as the present results and previous work in our laboratory (9) demonstrate, the time-course of the response of HPA axis are complex since post-morphine and post-stadol hormone levels can be increased, decreased or unchanged compared to control levels depending upon the dose, the time of sampling as well as the species variability (9,20,23,24,44).

REFERENCES

1- Briggs FN and Munson PL : Endocrinology, 57:205-219, 1955.

2- George R and Kokka N : Tissue Response to Addictive Drugs. DH Ford and DH Clouet, Spectrum, pp 527-540, New York, 1976.

3- Buckingham JC : Neuroendocrinology, 35:111-116, 1982.

4- Buckingham JC and Cooper TA : Neuroendocrinology, 38:411-417, 1984.

5- Suemaru S, Hashimoto K and Oka Z : Acta Med Okayama, 39:463-470, 1985.

6- Buckingham JC and Cooper TA : Neuroendocrinology, 44:36-40, 1986.

7- Buckingham JC and Cooper TA : Opioid Modulation of Endocrine Function-G Delitala, M Motta and M Serio, Raven Press, pp 81-87, New York, 1984.

8- Lotti VJ, Kokka N and George R : Neuroendocrinology, 4: 326-332, 1969.

9- EL Daly ES : J Egypt Soc Toxicol, 12:11-18, 1994.

10- Bertolini A, Poggioli R and Fratta W : Life Sci, 29:294, 1981.

11- Gianoulakis C, Drouin JN, Seidah NG, Kalant H and Chretien M : Eur J Pharmacol, 72:313, 1981.

12- Hollt Y, Emrich HM, Bergman M, Nedopil N, Dieterle D, et al : Endorphins and Opiate Antagonists in Psychiatric Research, NS Shah and A G Donland, Plenum Press, p 23, New York, 1982.

13- Hollt V and Haarmann I : Neuropeptides, 5:481, 1985.

14- Bradford M : Anal Biochem, 72:248-254, 1976.

15- Pechnick R, George R and Poland RE : J Pharmacol Exp Ther, 232:163-169, 1985.

16- Howlett TA and Rees LH : Ann Rev Physiol, 48:527-536, 1986.

17- Iyengar S, Kim HS and Wood PL : Brain Res, 435:220-226, 1987.

18- Lightman SL and Young WS : J Physiol, 403:511, 1988.

19- Hollt V, Przeewlocki R and Herz A : Life Sci, 23:1057-1066, 1978.

20- Martinez JA, Vargas ML, Fuente T, Rio-Garcia JD and Milanes MV : Eur Gen Pharmacol, 182:117-123, 1990.

21- Gibson A, Ginsburg M, Hall M and Hart SL : Br Gen Pharmacol, 66:164-166, 1979.

22- Borrell J and Borrell S : Neurosci Lett, 4:191-195, 1977.

23- Milanes MV, Rio-Garcia JD, Fuente T, Martinez JA and Vargas ML : Gen Pharmacol, 21:569-571, 1990.

24- Vargas ML, Martinez JA, Fuente T, Rio-Garcia JD and Milanes MV : Gen Pharmacol, 22:223-226, 1991.

25- Grossman A, Gailard RC, McCartney P, Rees LH and Besser GM : Clin Endocrinol, 17:279-286, 1982.

26- Hiroshige T, Fujieda K, Kaneko M and Honma K : Ann NY Acad Sci, 297:436-454, 1977.

27- French ED, Bloom FE, Rivier C, Guillemin R and Rossier J : Soc Neurosci Abstr, 4:408, 1978.

28- Gillham B, Beckford U and Insall RL : A McL Skelly, MT Jones, J Endocr, 90:201-210, 1981.

29- Buckingham JC : Br J Pharmacol, 75:27, 1982.

30- DeSouza EB and Van Loon GR : Endocrinology, 111:1483-1490, 1982.

31- Plotsky PM, Otto S and Sapolsky M : Endocrinology, 119:1126-1130, 1986.

32- Polkowska J and Przekop F : Acta Endocrinologica, 118:269-276, Copenhag, 1988.

33- Nikodijevic O and Maickel RP : Biochem Pharmacol, 16:2137-2142, 1967.

34- Guaza C, Torrellas A, Borrell JK and Borrell S : Pharmacol Biochem Behav, 11:57-63, 1979.

35- Wuster M, Schulz R and Herz A : Brain Res, 189:403, 1980.

36- McDonald RK, Evans FT, Weise VK and Patric RW : J Pharmacol Exp Ther, 125:241-247, 1959.

37- Kokka N, Garcia JF and Elliott HW : Brain Res, 39:347-354, 1973.

38- Munson PL : Narcotic and the Hypothalamus, E Zimmerman and R George, Raven Press, pp 361-372, New York, 1974.

39- Brandt MR, Korshin J, Hansen AP, Hunner L, Madsen SN, Rugg I and Kehlet H : Acta Anaesthesiol Scand, 22:400-412, 1978.

40- Abdel Raheem KA, Mossallamy NEl, Hassan R, Okasha S, Badawi M and Abdel Kader A : Egypt J Physiol Sci, 15:97-106, 1991.

41- Facchinetti F, Grasso A, Petraglia F, Parrini D, Volpe A and Genazzan A : Acta Endocrinocol, 105:149, 1984.

42- Mains RE, Eipper BA and Ling N : Proc Natl Acad Sci, USA, 74:3014, 1977.

43- Koening JL, Maltzer HY and Gudelsky GA : Neuroendocrinology, 43:611, 1986.

44- Evans CJ, Erdelyi E and Barchas JD : Psychorpharmacol Bull, 21:466-471, 1985.

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