Symposium on substance P

urednik Stern, Pavao

1961

Naučno društvo NR Bosne i Hercegovine

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NAUČNO DRUŠTVO NR BOSNE I HERCEGOVINE

POSEBNA IZDANJA
Vol. I

ODJELJENJE MEDICINSKIH NAUKA
Knjiga 1

Urednik

P. STERN
redovni član Naučnog društva NR BiH

SIMPOZIJUM
O
SUPSTANCIJI P

održan 9. i 10. VI 1961. god.

SARAJEVO
1961

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AND E. MÜLLER

NEUROHUMORAL CONTROL OF THE ANTERIOR PITUITARY GLAND

Developments of the last few years have made it increasingly clear that the nervous system may be considered a complex endocrine system. The view is now developing rapidly that neurons may be producers and releasers of a variety of active physiological agents known as neurohumors. Some nervous cells, termed »neurosecretory cells« (Scharrer, 1959) have carried the secretory activity to the point where they become morphologically distinguishable from other nervous cells.

Substances related to nervous activity which have been chemically identified are ACh, adrenaline, noradrenaline, 5-HT (serotonin) and three posterior pituitary peptides : vasopressin, oxytocin and arginine-vasotocin (Pickering and Heller, 1959; Sawyer, Munsick and Van Dyke, 1959). Neurohumors which are still waiting to be chemically identified are the factors which control aldosterone secretion and those which regulate the activity of the anterior pituitary.

Much evidence has accumulated in recent years to show that the central nervous system influences the secretory activities of the anterior lobe of the pituitary gland (Harris, 1955).

Although the question of innervation of the adenohypophysis is not settled, there is little evidence that important nervous connections exist between it and the brain; it is then likely that nerve fibres which arise in the hypothalamus liberate some humoral substance(s) into the capillaries of the primary plexus of the portal vessels at the level of the median eminence, and that this (these) substance(s) is (are) carried by the portal vessels to the *pars distalis* (Harris, 1955; Assenmacher and Benoit, 1958).

Identification of the ACTH releasing portal chemotransmitter has been claimed by several groups of investigators but the results are up to now conflicting.

A component of SP has been shown to induce ACTH discharge from the pituitary both »in vitro« (Guillemin, Hearn, Cheek and Housholder, 1957) and »in vivo« (Swingle, Parlow, Brannick and Barret, 1956) and then proposed as the possible chemotransmitter involved in the ACTH-releasing mechanism (Pernow, 1953).

Vasopressin (antidiuretic hormone, ADH) has also been proposed as the ultimate mediator of ACTH release; the possible role of this substance is supported by the following evidence: (1) the neurosecretory material stored in the neurohypophysis and containing ADH may be depleted by noxious stimuli which also induce ACTH release (Rothballer, 1953; Scharrer and Frandson, 1954; Kivalo and Rinne, 1960); (2) ADH and ACTH are discharged simultaneously after the exposure to stressful stimuli (Mirsky, Stein and Paulisch, 1954) and after the administration of several drugs (adrenaline, ACh, etc.) (Martini and Rovati, 1956; Casentini, De Poli and Martini, 1957); (3) neurogenic stimuli are much less active as ACTH releasers in neurohypophysectomized than in normal rats, if the operated animals are not given ADH (De Wied, 1960); (4) extracts containing the antidiuretic activity of the posterior lobe and synthetic antidiuretic hormones (lysine- and arginine-vasopressin) are effective in inducing ACTH release in normal animals (Martini and Morpurgo, 1955; Martini, De Poli and Curri, 1956; Casentini, De Poli, Huković and Martini, 1959; Rochefort, Rosenberger and Saffran, 1959), in hypophysectomized animals bearing a functional pituitary graft in the anterior chamber of the eye (Martini and De Poli, 1956; Casentini et al., 1959; Martini, De Poli, Pecile, Saito and Tani, 1959), in animals with hypothalamic lesions (McCann, 1957; Jorgensen and Nielsen, 1958; Jorgensen and Larsen, 1960), in neurohypophysectomized rats (Nowell, 1959) and in pharmacologically blocked animals; in pharmacological experiments hydrocortisone (Porter and Jones, 1956; McCann, 1957; Chauvet and Acher, 1959), 9-alpha-fluorohydrocortisone (Casentini et al., 1959), prednisone (Smelik and De Wied, 1958), prednisolone (De Wied and Mirsky, 1959), morphine (McCann, 1957; Smelik, 1959), nembutal-morphine (Guillemin, Nichols and Lipscomb, 1958; De Wied, Bouman and Smelik, 1958; Munson and Leeman, 1958) or chlorpromazine (Sevy, Ohler and Weiner, 1957) have been used; (5) injections of little amounts of arginine-vasopressin into the third ventricle of dogs produce a significant rise in 17-hydroxy-corticosteroid level in adrenal venous blood (Kwaan and Bartelstone, 1959); (6) natural and synthetic neurohypophysial hormones exhibit ACTH-releasing activity in cultures of hypophysial tissue (Saffran, 1959); (7) the ACTH releasing activity and the pressor activity of lysine-vasopressin are altered to the same extent following mild acid and alkaline hydrolysis, iodination and incubation with placental extracts (Sideman and Sobel, 1960); (8) adrenal corticoids inhibit the release of ACTH as well as the release of ADH (McCann, Fruit and Fulford, 1958; Giuliani, Martini and Pecile, 1960).

The present report will study »in vivo« the ACTH releasing activity of SP and of several natural and synthetic peptides with posthypophysial activities.

Methods

Rats of the Sprague-Dawley strain weighing 150—200 g were used. First of all the ACTH releasing effect of two samples of SP

(obtained through the courtesy of Prof. Gaddum and of Prof. von Euler) and of Pitressin, purified lysine-vasopressin, Pitocin and synthetic oxytocin was studied in normal animals by means of the adrenal ascorbic acid depletion method (Sayers, Sayers and Woodbury, 1948); details on the method employed are given in a previous paper of this laboratory (Casentini et al., 1959). Principles which were found active in this test and a few other synthetic peptides which became available in the last few years were then assayed for ACTH-releasing activity using a new method. In this method the release of ACTH is assessed by variations of concentrations of plasma free corticosterone in rats which had been previously injected intraperitoneally with 25 μg/100 g body weight of dexamethasone in order to block aspecific pituitary stimulation. The structure of the principles employed in these experiments is given in Fig. 1. The time schedule of these experiments was as follows:

hr 0 Dexamethasone 25 μg/100 g, intraperitoneally
hr 3.45 Nembutal 3 mg/100 g, intraperitoneally
hr 4.00 Peptides intravenously
hr 4.30 Decapitation and blood collection

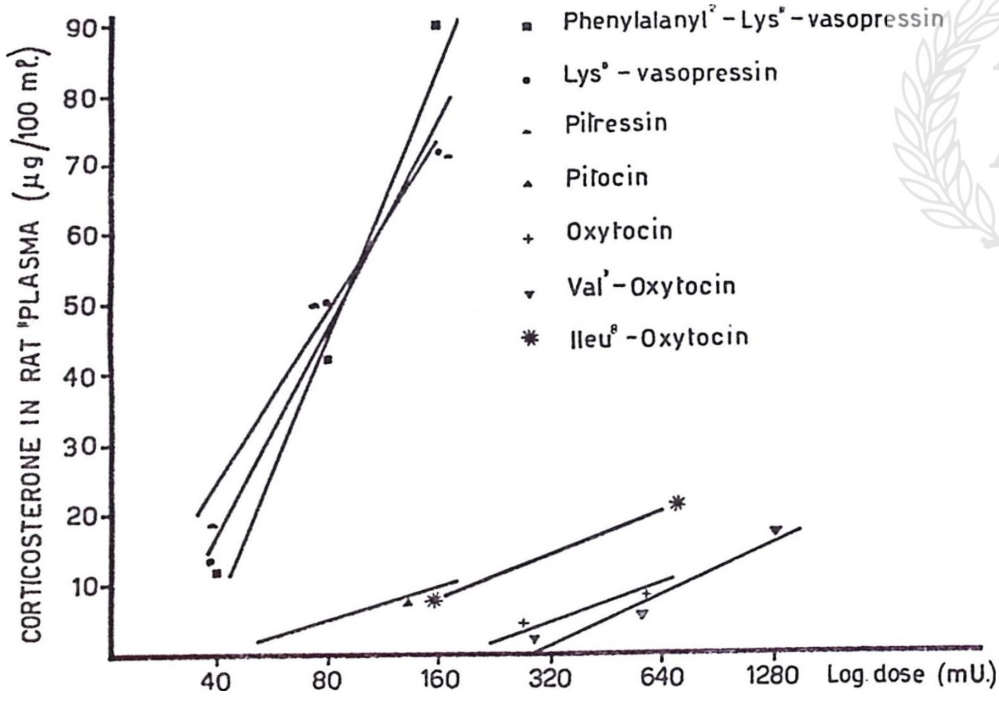

FIG. 1

Administration of dexamethasone has been shown not to interfere with corticosterone blood determinations, to induce a drop of corticosterone blood levels similar to that induced by hypophysectomy and to inhibit the stress induced ACTH-discharge (Giuliani, Martini, Mül-

ler and Pecile, 1961); the sensitivity to ACTH of the dexamethasone-treated animal has also been studied (Giuliani and coll., 1961).

Plasma free corticosterone determinations have been carried out according to the method of Silber, Bush and Oslapas (1958) as modified by Guillemin, Clayton, Lipscomb and Smith (1959).

Results

Table I shows that in normal animals the injection of Pitressin and of Pitocin is followed by a highly significant fall in adrenal ascorbic acid concentration ($P < 0.001$); lysine-vasopressin and synthetic oxytocin also have marked ACTH releasing activity in normal animals; two different samples of SP are completely ineffective in the ascorbic acid depletion test; this last result differs from those obtained by Swingle et al. (1956).

TABLE I

ADRENAL ASCORBIC ACID DEPLETION INDUCED BY VARIOUS PEPTIDES IN NORMAL RATS.

Treatment	No. of rats	Adrenal ascorbic acid concentration (mg/100 g adrenal) Mean ± S. E.	P (Fisher's table)
NaCl 0.9%	26	411 ± 8	
SP (horse) 10 U.	6	427 ± 43	
SP (cow) 10 U.	6	436 ± 28	
Pitressin 0.3 U.	12	302 ± 14	< 0.001
Pitocin 0.3 U.	23	358 ± 12	< 0.001
Lysine-vasopressin 0.3 U.	18	311 ± 8	< 0.001
Synthetic oxytocin 0.3 U.	23	358 ± 16	< 0.005

When the peptides were tested as ACTH releasers in dexamethasone-treated rats the following results were obtained (Fig. 2): Pitressin, synthetic lysine-vasopressin and phenylalanyl-lysine-vasopressin showed a considerable activity; a linear log-dose response relationship could be obtained with doses ranging from 40 to 160 mU.; the greatest activity was shown by the synthetic peptide phenylalanyl-lysine-vasopressin. By contrast Pitocin, synthetic oxytocin, valyl-oxytocin (Berde, Doepfner and Konzett, 1957) and isoleucyl-oxytocin (Berde and Konzett, 1960) were very poor ACTH releasers.

These results seem to indicate that the determination of blood corticosterone levels in the dexamethasone-inhibited rat offers a new approach for the »in vivo« study of ACTH-releasing activity of hypophysiotrophic substances.

Moreover the results obtained with this new test seem to give new support to the hypothesis that posterior pituitary principles may be involved in the physiological regulation of ACTH release.

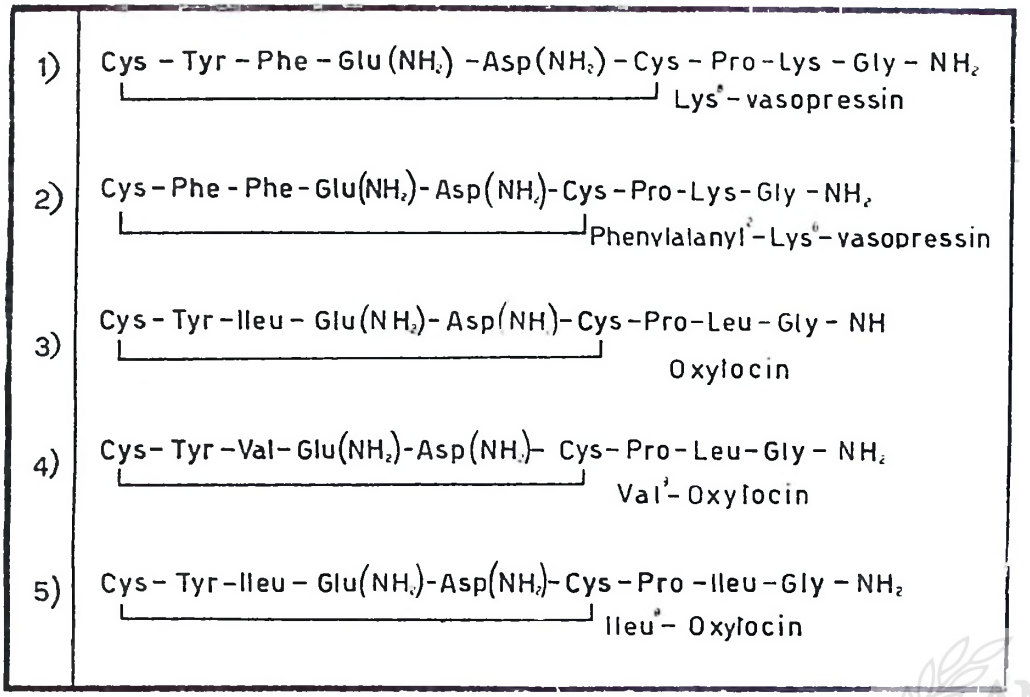

FIG. 2

It seems also worth mentioning that these experiments have clearly shown that also synthetic non-natural peptides may be very active ACTH releasers.

If we consider the acitivity for mg of the peptides used in the present report (which is 280 U./mg for lysine-vasopressin; Du Vigneaud, Bartlett and Jöhl, 1957), it becames evident that these peptides act in very little amounts, ranging from about 0.15 μg to about 0.60 μg. A similar degree of activity was found »in vivo« by Schally and Guillemin (1960) for the preparation they call CRF (Corticotrophin-Releasing Factor).

Similar results and conclusions were also reached by De Wied and Mirsky (personal communication) who have shown that oxypressin, a synthetic peptide which contains the cycle of vasopressin and the side chain of oxytocin, releases ACTH in rats, as measured by the adrenal ascorbic acid method.

A result similar to those reported in the present paper has also been obtained by Miller, Arimura and Dingman (1960) who have shown that lysine- and arginine-vasopressin produce marked increases in plasma corticosterone levels in prednisolone-treated rats and that two unidentified compounds free of vasopressor activity were also devoid of ACTH-releasing activity.

The negative results generally obtained with oxytocin are in agreement with the results of Rinne, Kivalo and Lahtinen (1959) who

have shown that oxytocin is not active in the adrenal ascorbic acid test after prednisolone administration.

Summary

The question whether the hypothalamo-hypophyseal pathway is nervous or humoral in nature has been widely discussed in recent years. The existing evidence points to a humoral rather than a nervous mechanism, and the hypothalamic-hypophyseal portal system has received much attention as the probable pathway through which a chemical transmitter could pass from the median eminence to the adenohypophysis.

Identification of the ACTH-releasing portal chemotransmitter has been claimed by several groups of investigators but the results are up to now conflicting. Two different samples of SP, assayed on the adrenal ascorbic acid depletion test in the rat, have shown to be completely ineffective as ACTH releasers; on the other side Pitressin, Pitocin, lysine-vasopressin and synthetic oxytocin were highly active in this test. Pitressin, synthetic lysine-vasopressin and phenylalanyl-lysine-vasopressin (a synthetic analogue of vasopressin) showed a considerable ACTH-releasing activity when tested in a new method of assay based on corticosterone blood determinations in dexamethasone inhibited rats. By contrast Pitocin, synthetic oxytocin, valyl-oxytocin and isoleucyl-oxytocin (two synthetic analogues of oxytocin) were very poor ACTH releasers in this test.

NEUROHUMORALNA KONTROLA PREDNJEG REŽNJA HIPOFIZE

U toku posljednjih godina mnogo se diskutiralo o pitanju da li je priroda hipotalamo-hipofiznog puta humoralna ili nije. Prikupljeni podaci više govore u prilog jednog humoralnog nego nervnog mehanizma pa je hipotalamo-hipofizni portalni sistem pobudio veliku pažnju kao mogući put za prolaz nekog kemijskog transmitora od središnje izbočine tub. ciner. ka adenohipofizi.

Više grupa istraživača tvrdilo je do sada da su identifikovale portalni kemotrasmitor koji oslobađa ACTH, no dosadašnji su rezultati protuslovni. Pri ispitivanju pražnjenja askorbinske kiseline iz štakorove nadbubrežne žlijezde, pokazalo se da su dva različita primjerka SP potpuno neefikasni za oslobađanje ACTH; pitresin, pitocin, lizin-vazopresin i sintetski oksitocin, naprotiv, bili su vanredno aktivni u istom testu. Pitresin, sintetski lizin-vazopresin i fenilalanil-lizin-vazopresin (jedan od sintetskih analoga vazopresina) pokazali su znatnu aktivnost i pri ispitivanju jednom novom metodom, zasnovanom na određivanju kortikosterona u krvi štakora tretiranih deksametazonom. Za razliku od navedenih spojeva, pitocin, sintetski oksitocin, valil-oksitocin i izoleucil-oksitocin (dva sintetska analoga oksitocina) vrlo su slabo oslobađali ACTH u novom testu.

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DISCUSSION

VOGT: Do you think that the amounts of vasopressin released under physiological circumstances are sufficient to release ACTH, or do you think as do Guillemin and Saffran, that the natural corticotrophin releasing factor is much more potent?

MARTINI: We think that, as it has been pointed out by Mirsky, the amounts of vasopressin released under physiological circumstances are sufficient to release ACTH; our experiments do not exclude, of course, that other molecules related to vasopressin could also be of importance for the nervous regulation of ACTH secretion.

STERN: Milin and I have some evidence that SP could release ACHT. This agrees with the views of some other authors. SP markedly depresses the C-vitamin content of the suprarenal gland.