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COMMENTARY

THE RADIOIMMUNOASSAY OF NEUROPHYSINS AS A TOOL FOR THE POSTERIOR PITUITARY INVESTIGATION

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1. INTRODUCTION

1.1. *The posterior pituitary hormones*

The posterior pituitary gland is a part of the central nervous system: it is in continuity with the anterior part of the hypothalamus. It contains two major hormones of low molecular weight (1100) and similar amino acid composition (nonapeptides) namely vasopressin and oxytocin. They are both synthetized within the paraventricular and supraoptic nuclei and they migrate toward the posterior pituitary where they are stored within the neurosecretory granules. This process was first described by Scharrer and Scharrer who named it neurosecretion [1]; hence the posterior pituitary which was previously considered as a site of synthesis of the hormone was at that time recognised as the locus of hormonal storage before the release occurs.

Antidiuretic hormone. Antidiuretic hormone (ADH) acts at the apical part of the distal renal tubules where it permeabilyses the cells and permits the entry of water along the medullar gradient thus allowing for the concentration of urine. In absence of ADH a very large quantity (up to 25 l./day) of diluted urine is excreted: thus this hormone plays a major role in water homeostasis. ADH also possesses a vasopressor activity, which was its first known biological action, however this action is observed in man or animals for doses which are far larger in excess than physiological levels of circulating hormones: the vasopressor activity is now generally considered as a pharmacological one, unrelated to the physiological, antidiuretic action.

Oxytocin. Oxytocin induces the contraction of myoepithelial cells of the mammary gland and is therefore responsible for the milk ejection reflex within the few minutes following mechanical stimulation of the nipple. It is also active on the uterine muscle and could play a role during parturition and expulsion of the foetus during parturition. Its exact physiological importance in that field is however unknown even if synthetic oxytocin is often used by clinicians to induce labour in woman (see a review of endocrine function in labour in [2]). Moreover, oxytocin is also found in the male where its role is unknown.

In addition to their well established action, ADH and oxytocin could also share other biological properties. Since both hormones are found in high levels in the antehypophysial portal blood vessels [3] and since injection of ADH or oxytocin may induce a release of some anterior pituitary hormones [4] it has been postulated that the posterior pituitary peptides could be involved in the control of anterior pituitary function mainly corticotrophic (ACTH) and gonadotrop (FSH and LH). Moreover, small peptides including vasopressin, could be physiologically involved in acquisition and maintenance of some behavioral patterns in animals. It has been demonstrated that vasopressin is specifically related to the consolidatory phase of the memory by de Wied and co-workers (see review in [5]).

The physiological stimuli for ADH release are hypovolemia and blood hyperosmolality: osmolality is more important than volemia for the day to day regulation of hormonal release [6]. However induced or spontaneous hypotension induces a major neurohypophysial activation in normal individuals [7]. Psychological stress induces a decrease of oxytocin [8] and ADH [9] release: this could be due to a direct impact on the hypothalamus through the corticohypothalamic pathway or to an increased level of norepinephrine which is known to inhibit neurohypophysial release. Most pharmacological substances which influence cerebral metabolism also modulate neuropituitary secretion. Hence alcohol [10], chlorpromazine and reserpine [11], oxylorphan and butarphanol (two narcotic antagonists) [12] inhibit ADH release while barbiturates [13] and nicotine [14] stimulates it *in vivo*. *In vitro*, urethane potentiates oxytocin release while pentobarbitone inhibits it [15]. Lastly cysteamine and cystamine (given at radioprotective dose) as well as whole body exposure to X-ray stimulate the neurosecretion in rat [16] suggesting that neuropituitary plays a role in the stress reaction of mammals to ionizing radiations.

In view of the different known or hypothetical biological actions of the posterior pituitary peptides, it is self evident that it is important to accurately appreciate the hormone release. The indirect index of ADH or oxytocin release (diuresis, osmolality, milk ejection) are non-specific while the bioassay are hampered by a lack of specificity and sensitivity. The direct radioimmunoassay of the hormones developed in blood and urine during the last 10 years are highly specific and very sensitive; they however suffer from technical difficulties due to the small molecular weight of the hormones and their use in routine practice is inconvenient (see review in [17]).

1.2. *The neurophysins*

Within the neurosecretory granules, vasopressin and oxytocin are non-covalently bound to high molecular weight carriers. The whole complex forms the

so-called "Van Dijke protein". Acher *et al.* [18] first named "neurophysin" the carrier substance of a molecular weight of approximatively 10,000; it can be dissociated from bioactive hormone under mild conditions of extraction (increasing or decreasing pH, increasing salt concentration).

There are at least two types of neurophysins in most animal species. The amino acid composition and molecular weight are very similar in the different neurophysins of the same species and also in various neurophysins of different species [19]. They can be differentiated through their electrophoretic mobilities and as it is used in enzymology, the more anodic form is called I and the less anodic form is named II. Both types of neurophysins can reversibly bind vasopressin and oxytocin *in vitro* and until recently, it was questioned whether there is a specificity *in vivo* of association between one neurophysin and one hormone. Complete data about the physicochemical characteristics of neurophysins can be found in recent reviews [20, 21]. Another debate arises as to the fate of the neurophysins at the time of hormonal release: according to some authors, free hormone diffused across the capillary membrane while neurophysins were degraded within posterior pituitary. According to other research workers, there is histological evidence that the release mechanism is exocytosis, as it is the case for many other hormonal systems (Fig. 1).

If that were the case then neurophysins has to be detected in the peripheral blood and their levels must increase at the time of hormonal release.

Indirect evidence for the presence of neurophysins in the blood was first provided in 1968 by Ginsburg and Jayasena [22] who extracted an "immunoreactive like neurophysin" from peripheral blood in the pig and by Fawcett *et al.* [23] who demonstrated the presence in the blood of a substance of a molecular weight similar to that of neurophysins during hemorrhage in dog. In 1969, we developed a radioimmunoassay for human neurophysins [24] and demonstrated variation of their blood levels in physiopathological conditions; this work was soon followed by results from Cheng and Friesen [25] and Robinson *et al.* [26] reporting the measurement of plasma neurophysins in rat, pig and ox.

Hence, at that time it appeared that assays for neurophysins which are methodologically more simple and reliable than that of active nonapeptides, could be suitable for the routine investigation of neuropituitary function. We will review here the major results obtained by the radioimmunoassay (R.I.A.) of neurophysins in the human and in animals and later emphasise the limits to the interpretation of the data obtained with this method.

2. THE RADIOIMMUNOASSAY OF NEUROPHYSINS

2.1. *The methodology of the assays*

Reviews of the literature comparing the methodological aspects of ADH and oxytocin R.I.A. on the one hand and of neurophysins on the other hand have been recently published [17, 27].

2.2. *Simultaneous release of hormones and neurophysins*

Although demonstration of the presence of neurophysins in the blood was a good argument in favour of their release together with the hormones, the strongest evidence for a simultaneous release was only given by the demonstration of a concomitant fluctuation of the levels of both hormones and its carriers in conditions of stimulation for posterior pituitary hormones release. In Table 1, we have quoted the works in which neurophysins and hormones were simultaneously measured in various experimental conditions. In all the physiological and experimental conditions tested until now; a parallelism between the variations of neurophysins and of hormones has been demonstrated. To illustrate this, we show in Fig. 2 the mean blood levels of vasopressin and of I.R.N. in the course of an i.v. nicotine test in normal man, a pharmacological activation used in clinical practice.

Those data firmly suggest that hormones are released through a mechanism of exocytosis and also

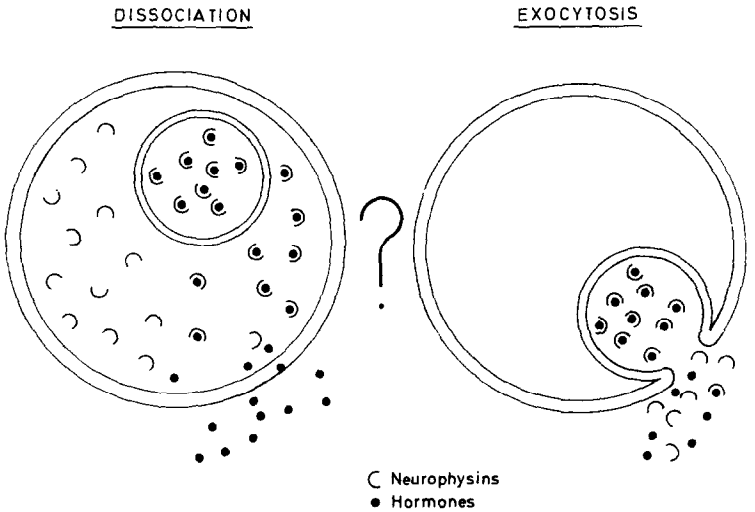

Fig. 1. Schematic representation of a neurosecretory granule within the neurohypophysis. Are the hormones alone released by a diffusion process or are they released together with neurophysins through an exocytosis mechanism?

Table 1. Experimental works where neurophysins and hormones were measured which evidenced a concomitant fluctuation of the two groups of peptides

Experiment	Assay used	Refs
In vitro. Stimulation of rat posterior pituitary	R.I.A. for I.R.N. and bioassay for oxytocin	[28]
In vitro. Stimulation of rat posterior pituitary	R.I.A. for I.R.N., A.D.H. and oxytocin	[29]
In vivo. Hemorrhage in goat	R.I.A. for I.R.N. and A.D.H.	[30]
In vivo. Parturition in goat	R.I.A. for I.R.N. and oxytocin	[31]
In vivo. Effect of estrogens on posterior pituitary content in rat	R.I.A. for I.R.N., A.D.H. and oxytocin Bioassay for oxytocin	[32]
In vitro. Stimulation of pig posterior pituitary	R.I.A. for specific pN_pI and pN_pII R.I.A. for A.D.H. and oxytocin	[33]
In vivo. Hemorrhage in pig	R.I.A. for specific pN_pI and pN_pII and A.D.H.	[33]
In vivo. Effect of dehydration on posterior pituitary content in rat	R.I.A. for I.R.N., A.D.H. and oxytocin Bioassay for oxytocin	[34]
In vivo. Effect of inhaling cigarette smoke in the human	R.I.A. for N.S.N. and A.D.H.	[14]
In vivo. Effect of nicotine injection in the human	R.I.A. for I.R.N. and A.D.H.	[35]

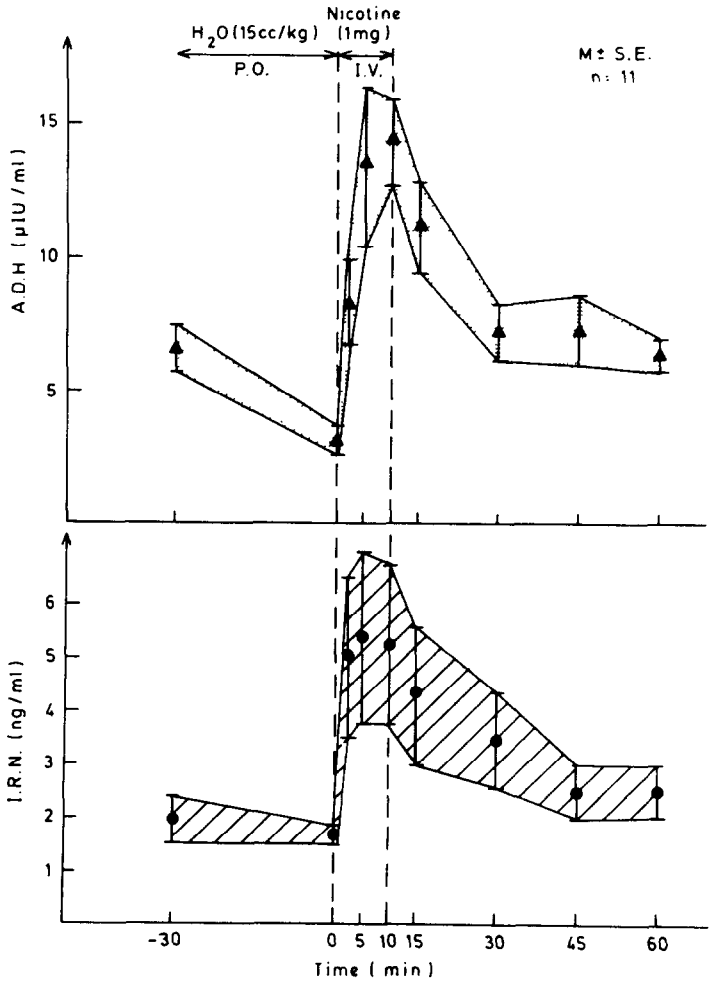

Fig. 2. Blood levels (antecubital vein) of vasopressin (ADH) and of immunoreactive neurophysins (I.R.N.) in the course of a water load test (15 ml/kg) followed by an i.v. injection of nicotine in 11 individuals with normal posterior pituitary function. There is a rapid, parallel, increase of hormone and its carrier protein in the peripheral circulation; the relationship between the increase of both substances is 0.87 (Legros *et al.*, 1977) [35].

Table 2. Experimental works where the specific release of one neurophysin without a release of the other has been demonstrated

Demonstration	Refs
Increase of bN_pI without a constant increase of bN_pII during parturition in the cow	[26]
Specific release of bN_pI without a release of bN_pII during suckling or milking in the cow	[36]
Specific release of bN_pII with only minor changes, if any, of bN_pI during arterial or venous hemorrhage in the cow	[37]
Specific increase of pNp I during hemorrhage in pig	[33]
Specific release of N.S.N. during inhalation of cigarette smoke and of E.S.N. following administration of exogenous estrogens in the human	[38]
Specific and time course independant release of bN_pI and bN_pII at various stages of parturition in the cow	[39]

indicate that the assay for neurophysins could probably be used to test the vasopressin and oxytocin release.

2.3. *Specific release of different types of neurophysins*

As mentioned in the introduction, *in vitro* physicochemical studies were unable to distinguish a specific "vasopressin–neurophysin" and an "oxytocin–neurophysin" in various animal species. The specific radioimmunoassays for each neurophysin firstly described by Robinson *et al.* [26] have now enabled some authors to demonstrate that it is possible to induce *in vivo* a specific release of one neurophysin without a release of the other according to the stimulus used. In Table 2, we have quoted the different studies which demonstrate a specific release of different neurophysins in various animal species and in the human. Individual examples of specific release of bN_pI and/or bN_pII in parturition, a physiological condition known to evoke a release of oxytocin or of both hormones is given in the Fig. 3 [39].

Hence in bovine N_pI is liberated in condition of release of oxytocin and N_pII in condition of release of ADH. This specificity of association between one neurophysin and one hormone has now been confirmed by immunohistochemical studies realised on the ox hypothalamus [40, 41]. In the pig, ADH seemed to be associated with pNp I [33]. The results are less clear in the human: we have brought data [42] indicating that within the neurohypophysis hN_pI is associated with vasopressin and hN_pII with oxytocin. This is confirmed in a preliminary clinical work studying the neurophysins and hormonal contents of four lung tumours secreting vasopressin and/or oxytocin (Schwartz–Bartter syndrome) [43]. On the other hand, Robinson described two types of immunoreactive neurophysins in the human; one of which (Estrogens Stimulated Neurophysin, E.S.N.) is increased under the influence of exogenous estrogens and the other (Nicotin Stimulated Neurophysin, N.S.N.) is constituted of three substances with different electrophoretic behavior but sharing similar immunogen. All three are released in a majority (about 60 per cent) of volunteers following inhalation of cigarette smoke [38, 44]. Although there is a significant relationship between the increase of N.S.N. and of vasopressin during inhalation of cigarette

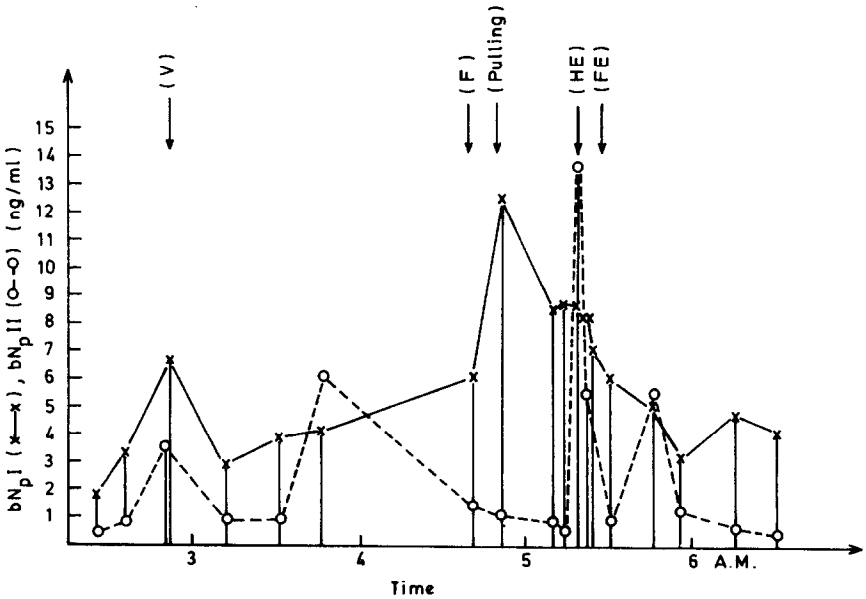

Fig. 3. Blood levels of neurophysin I (bN_pI) and neurophysin II (bN_pII) during iterative blood sampling in one jugular vein in one cow during a dystocic parturition. V: vaginal examination; F: dystocic presentation of the foetus; Pulling: manual correction of the malpresentation; H.E.: expulsion of the head; F.E.: expulsion of the calf. One observes a plateau of high bN_pI level lasting at least 1 hr; it is important to note 4 surges of bN_pII, 3 of which are independent from bN_pI release. This data demonstrates the separate release of both neurophysins as evidenced through their specific radioimmunoassays (after Legros *et al.*, 1976) [39].

smoke [14], no definite proof has until now been given that N.S.N. specifically represents the neurophysin(s) related to ADH and the E.S.N. the neurophysin(s) related to oxytocin within the human neuropituitary. It is then difficult to make a comparison between hN_pI, hN_pII, E.S.N. and N.S.N. at the present time (see 2.4.).

The different data mentioned here are in favour of a specific association between one neurophysin and one hormone in the hypothalamus. Moreover the results obtained in the lung tumour suggest that the genetic control of the biosynthesis of each hormone and its associated neurophysin is similar or identical. Further, they indicate that the separate radioimmunoassay for *each* neurophysin can be used to study the *specific* release of ADH and oxytocin.

2.4. *The results obtained in human physiology and pathology*

As previously mentioned (see 2.3.), there is no neurophysin standard available for the radioimmunoassay in the human. Therefore since there is a great similarity between animal and human neurophysin some authors use bovine or pig neurophysins as reference antigen [24, 45]; others use human neurophysins as the reference and antibodies obtained against animal neurophysins [46]. To date only Robinson described an homologous radioimmunoassay system using human neurophysins as immunogens and references [38]. Those differences make it difficult to compare data given by different groups of authors and can sometimes induce confusion. Therefore we have listed in Table 3 comparisons between the radioimmunological systems used together with the blood levels measured in normal, hydrated individuals. One can see that although the immunologic systems are quite different, the levels found are very similar.

Neurophysin blood levels have also been studied in various physiological and pathologic conditions well known to induce modifications of neurohypophysial function. They varied in the expected way in most of them: thus the neurophysins levels have been found to be *elevated* during dehydration, muscular exercise, spontaneous or induced hypotension, lactation, ectopic cancer secretion, renal tubular insensitivity to ADH and *low* during water load test, idiopathic benign arterial hypertension, chlorpromazine therapy. The majority of the results has been reviewed recently [43, 44, 47]. Salt load which is thought to stimulate ADH release induces only a weak, if any, increase of neurophysins blood levels [45, 47] while parturition (a putative condition of oxytocin release) induces only minor changes of peripheral neurophysins levels in the woman [43, 48]. It is likely that in those last two conditions the neurohypophysial stimulation is less than it was presumed before using indirect index of hormonal release.

Besides those classical conditions, neurophysin R.I.A. also permitted description of changes in neurohypophysial function in some physiological or pathological states during which such modifications were suspected but not proved: an increase of neurophysin blood level has been demonstrated during gestation [24, 46, 48], following estrogen injection (see Fig. 4), and in patients suffering from hepatic cirrhosis [51]. The physiological meaning of the circulating Estrogen Stimulated Neurophysin (E.S.N.) is still unknown. According to some personal results, this increase could be related to an increase mainly of ADH secretion while according to Robinson, this increase could presumably be the reflexion of an oxytocin release. Cyclic modification of neurophysin blood levels related to the modification of endogenous estogen blood levels has been found in the normal woman [52], confirming data obtained in the monkey [53]. Lastly, basal [47] and estrogen stimulated neurophysins [54] have been found low after the age of 50 both in man and woman. This observation suggests that the neurophysin blood levels could be used for future studies of aging of the hypothalamus.

Table 3. Different radioimmunoassay systems used for neurophysins estimation in the human with the values obtained in normal, hydrated, individuals

Refs	Basal Levels (ng/ml)	Immunological system used
[49]	Man : 1.26 ± 0.21 Woman: 2.24 ± 0.10	Heterologous bovine assay system appreciating all the circulating neurophysins (I.R.N.). Starch gel electrophoresis reveals 2 major components named hN_pI and hN_pII and 2 minor forms which are unconstantly found in the serum which are labelled temporary hN_pII^* and hN_pI^* (see discussion in [43]
[46]	Man and woman: 2 ± 1 ng/ml	Homologous assay system using purified hN_pI; there is an incomplete cross-reaction with hN_pII
[38]	Woman: 1.1 ± 0.7 Man : 1 ± 0.7	Homologous assay system which appreciates one single neurophysin liberated under the influence of exogenous administration of estrogens (Estrogens Stimulated Neurophysin, E.S.N.). This substance is similar to hN_pI
[38]	Woman: 0.9 ± 0.2 Man : 0.6 ± 0.3	Homologous assay system appreciating 3 substances with a similar immunologic behavior but which can be differentiated by electrophorectic properties. They are released under the influence of inhaling cigarette smoke (Nicotin Stimulated Neurophysin, N.S.N.). They seem to correspond to hN_pII, hN_pII^* and hN_pI^*
[45]	Man : 3.7 ± 1.53	Heterologous bovine assay system specific for bN_pI. No opinion about the human equivalent
[45]	Man : 5.87 ± 1.87 Woman: 4.13 ± 1.19	Heterologous bovine assay system specific for bN_pII. No opinion about the human equivalent

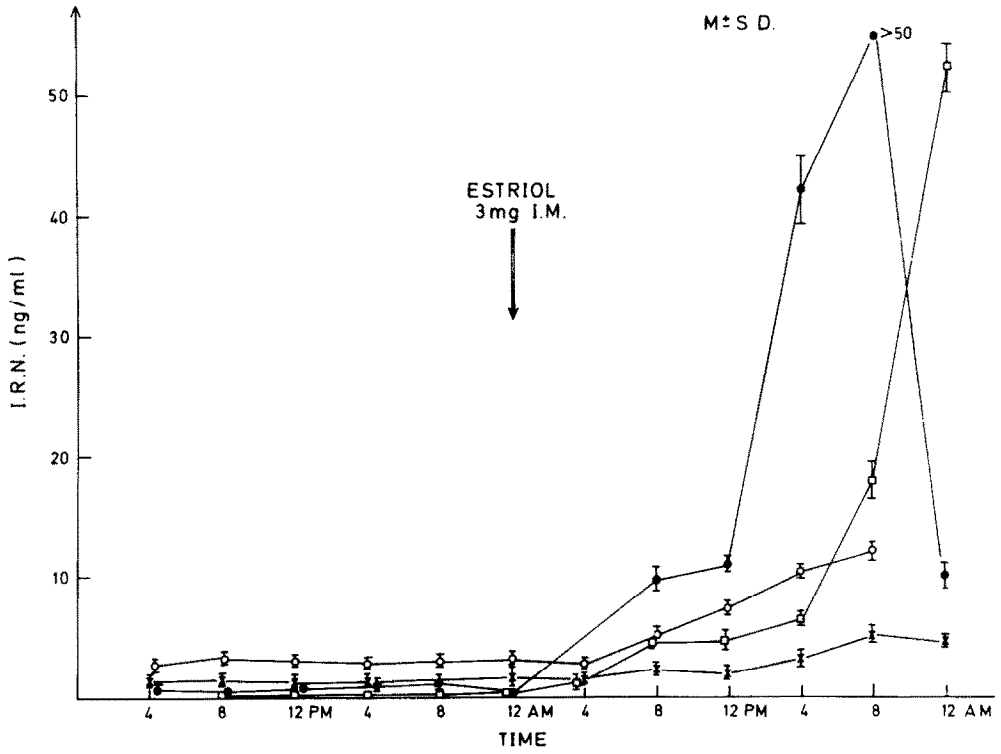

Fig. 4. Blood levels of immunoreactive neurophysins (I.R.N.) in samples taken every 4 hr for 48 hr. After a 24 hr control period, Estriol (3 mg) was injected i.m. in the 4 volunteers. All the assays were run in triplicate within the same assay series. A clear cut increase of neurophysins following the estrogen injection is to be observed (after Legros and Franchimont, 1970) [50].

3. THE VALUE OF NEUROPHYSINS R.I.A. FOR POSTERIOR PITUITARY INVESTIGATION

As we have shown the radioimmunoassay for neurophysins brings two arguments of fundamental importance to the basic knowledge of posterior pituitary function. These are:

(a) a *quantification* of the release during hormonal stimulation which, in conjunction with histological data, strongly supports the assumption that exocytosis is the release mechanism of vasopressin and of oxytocin.

(b) the *proof* that *in vivo* one hormone is specifically bound to one neurophysin in the normal hypothalamus and presumably in the neoplastic tissues. This gives arguments in favour of the unicity of the genetic control of the biosynthesis of the hormone and its carriers.

The radioimmunoassay for circulating neurophysins thus appears as a useful tool to appreciate precisely and directly the neuropituitary regulation. There is however some limits to the interpretation of the datas obtained through the neurophysin R.I.A. which have to be discussed.

3.1. *The serum levels of neurophysins in basal condition*

It is generally accepted in medicine and particularly in endocrinology, that a basal hormonal level rarely permits to analyse the integrity of a regulatory mechanism. Dynamic tests studying the hormonal reserve of the gland and the possibility of modulating the hormonal secretions are therefore most useful in clinical investigation. In addition to this general limitation to the interpretation of the basal level, there are some limits which are particular to the investigations using radioimmunoassay of neurophysins:

(a) Although some authors claimed that neurophysins share some biological activity (lypotrophic, gonadotrop, chronotrop, see review in [55]), it is well established that the carriers are devoided of any vasopressor, antidiuretic or oxytocin action. Moreover the weak affinity constant for vasopressin or oxytocin at physiological pH (order of magnitude 10^{-3} l./mole) makes unlikely the persistence of the hormone binding in the blood stream. Pliska *et al.* [56] however defined recently a neurophysin population with higher affinity constant (order of magnitude 10^{-6} l./mole) but to our knowledge, this has not been confirmed. Hence, a basal blood level of neurophysins does not reflect any biological activity. This is also confirmed by the comparison of the blood levels of neurophysins and of bioactive hormones. Indeed, if one loosely assumed a neurophysin molecular weight of 10,000 and a nonapeptide molecular weight of 1100, an association of 1 mole of neurophysin per-mole of nonapeptide would lead to circulating blood levels of A.D.H. around 120 pg/ml. This is far in excess to what is found by bio- or radioimmunoassay (from 0.1 to 9 pg/ml).

(b) The metabolism of neurophysins and hormones are similar: the degradation occurs mainly in the kidney [57] and presumably in the liver. However, the half lives of the carriers and the hormones are slightly

different (5.4 ± 0.82 min for vasopressin and 9.5 ± 1.4 for neurophysins in the dog) [57] which may e.g. account for a discrepency between a long-lasting high neurophysin blood level and a decrease of vasopressin in some acute, long-lasting experiments.

(c) Besides the two major circulating neurophysins some "minor" types have been described in various animal species: bovine [58], porcine [59], human [38, 43]. Those minor forms could be precursors or degradation products of the native neurophysins and then could constitute a reflection of nonapeptide release. They however could also be carrier substances for neurohypophysial peptides other than vasopressin and oxytocin. Let us recall that a natriuretic substance [60], coherin [61] and a second oxytocin hormone [62] have been isolated from posterior pituitary from various animal species. Moreover one should remember that in 1960, Murray and Miller isolated a substance from the posterior pituitary gland of rats which potentiates the analgesic activity of some morphine type agents [63]. Very recent data bring arguments in favour of some relationship between neurohypophyseal hormones and morphine dependance [64] and/or endogenous endorphins [65]. Finally, Parry and Livett [66, 67] described immunoreactive neurophysins probably unrelated to neurohypophysial hormone in the sheep median eminence. If those data are confirmed, it means that modifica-

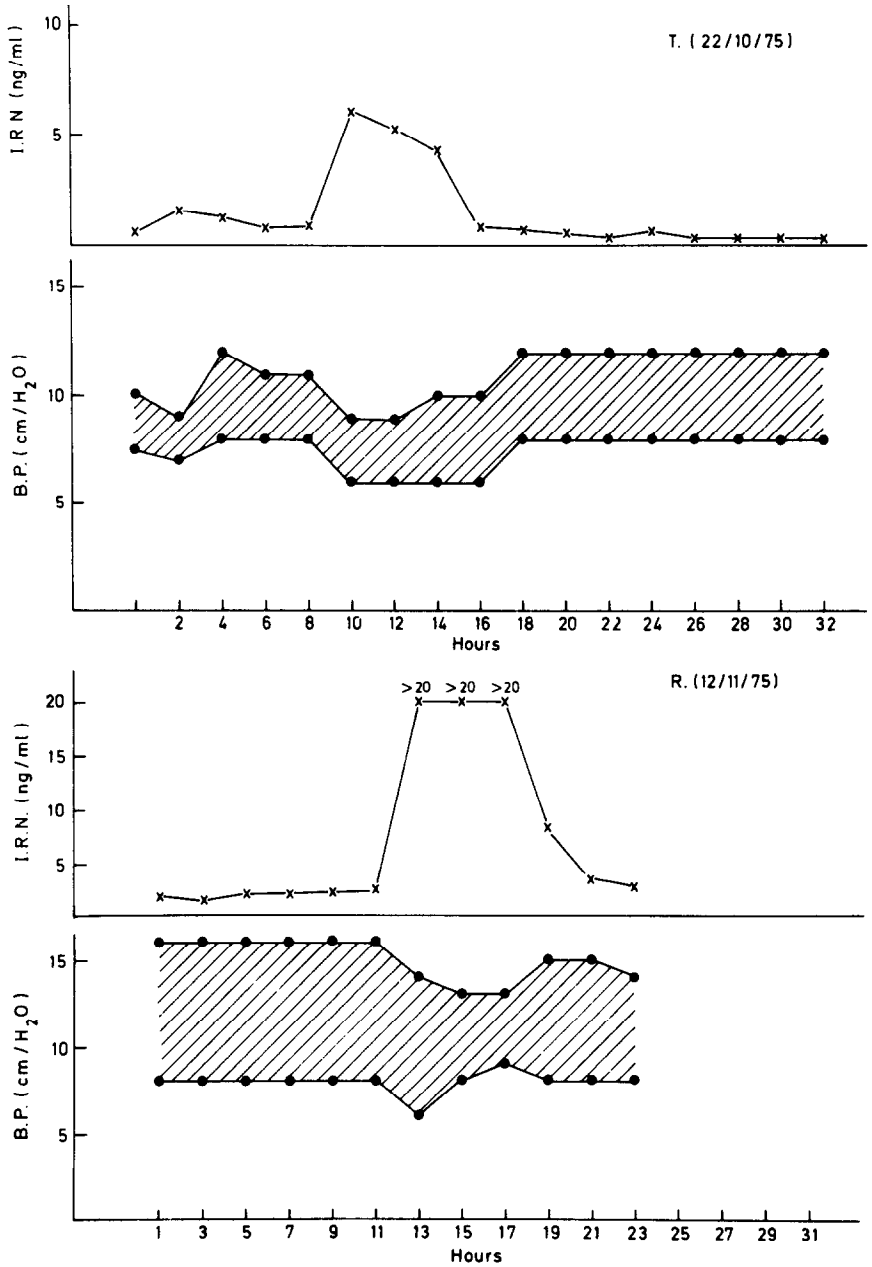

Fig. 5. Basal blood level of immunoreactive neurophysins (I.R.N.) in the antecubital vein in two patients whose blood pressure (B.P) was monitored each 2 hr during the day following a specific antehypophysial adenomectomy. One observes that minor decrease of blood pressure induces clear cut increase of I.R.N. levels (unpublished results).

tions of neurophysins serum levels can occur independently of variations in the release of ADH and of oxytocin.

(d) One has also to know that patients treated, even for a very short time, with crude posterior pituitary extracts developed circulating antibodies against neurophysins which could interfere with the assay system [43, 45, 68]. Hence, we described in 1973 [69] a "bN_pI-like substance" in the sera from some patients suffering from central diabetes insipidus but we were unable to confirm this latter. Those false results were artefacts due to the presence, in minute amounts, of circulating antibodies directed against bN_pI which interfered in the assay system (see discussion in [27]). The interpretation of a basal I.R.N. level in patients suffering from diabetes insipidus is then only valuable if no treatment with crude posterior pituitary extract was given, even for a short time, in the years preceding the blood sampling. Antibodies to vasopressin susceptible to interfere with the radioimmunoassay of the hormone, have also been described in such patients but with apparently a lower frequency [70].

(e) Lastly one has to be cautious in the interpretation of high levels which could be due to a very transient activation of the neurohypophysis due to some vagal effects or fainting tendency frequently found during blood sampling [7]. This effect of small changes in blood pressure on neurophysin level is illustrated in Fig. 5 in two patients who underwent antehypophysial selective adenomectomy and in whom I.R.N. levels and blood pressure were regularly monitored. The absence of such side effect at the moment of the venepuncture has then to be controlled carefully before the interpretation of neurophysin (or of ADH) blood level.

In spite of the above limitations, a basal neurophysin blood level, coupled with the estimation of the osmolality of the same sample, can give useful information in clinical practice as e.g. in dehydrated patients suffering from central diabetes where neurophysins levels are abnormally low when compared to the abnormally high blood osmolality and in patients suffering from a paraneoplasic hypersecretion of ADH where neurophysin levels are high or normal despite the blood dilution [43]. Moreover preliminary results obtained in rats indicate that basal neurophysin levels can be used as an index of the hypothalamic impact of some psychoactive drugs. In Fig. 6 the basal I.R.N. serum levels in 4 groups of 5 rats submitted to barbiturates (a drug known to induce neurohypophysial release), to fluanisone (a butyrophenone which shares an inhibitory hypothalamic action) or to the two combined substances, are shown. One sees that neurophysins vary in the expected way under the influence of barbiturates and fluanisone and that there is a competition between both drugs at the hypothalamic neurohypophysial level. These data support the assumption that the neurophysin R.I.A. will be useful in future psychopharmacological studies.

3.2. *The serum levels of neurophysins in dynamic tests*

Variations of neurohypophysial function can be studied during inhibitory tests (water load, alcohol injection or ingestion, drugs) or during stimulatory tests (dehydration, nicotin infusion, cigarette smoke inhalation, hypotension).

We remain sceptical about the results obtained during inhibitory tests, mainly because of the different half-lives of circulating hormones and of neurophysins (see 3.1). Moreover the decrease of neurophysin blood levels can be independent of the neurohypophysial secretion. The metabolic clearance rate of neurophysins varies with the renal glomerular filtration rate (G.F.R.): the greater the G.F.R., the greater the neurophysin metabolic clearance rate (Legros and Nizet, unpublished results). During a water load test a decrease of neurophysins blood levels could be partially secondary to the increase of the renal G.F.R.

We are more confident in the results obtained during acute stimulatory test; a putative release of an extragranular pool of "free" hormones without release of neurophysins, has, to our knowledge, never been proved at the present time (see discussion in [71]). The release of neurophysins without the liberation of ADH or oxytocin is less probable although the demonstration of immunoreactive neurophysins in extraneurohypophysial cerebral zone (median eminence [63, 64], pineal gland, target organs [72]) and the identification of new neurohypophysial bioactive small peptides presumably bound to neurophy-

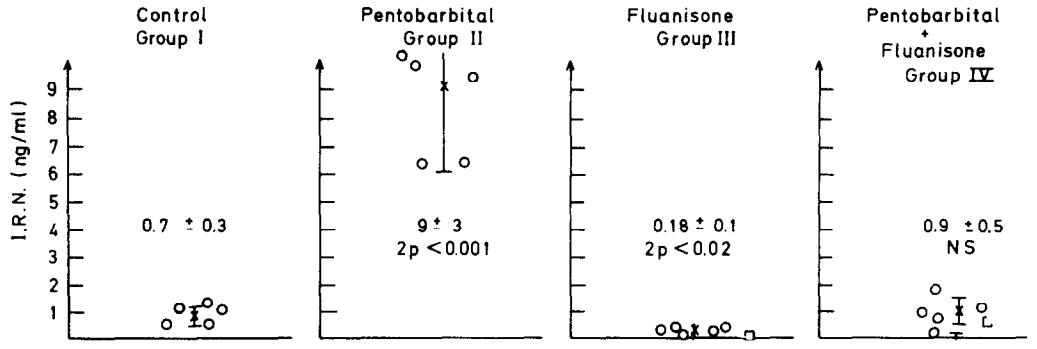

Fig. 6. Blood levels of immunoreactive neurophysin (I.R.N.) in 4 groups of 5 rats treated 15 min before decapitation by 0.5 NaCl 0.9% (control), pentobarbital (5 mg/100 g intraperitoneally) (group 2), fluanisone (1 mg s.c.) 30 min before sacrifice (group 3) or both drugs given respectively 15 min and 30 min before sacrifice (group 4). I.R.N. increases following barbiturates treatment; this activation is blocked by the previous injection of the butyrophenone. Fluanisone itself induces a significant decrease of basal I.R.N. serum levels (unpublished results).

sins-like substance (see 3.1) have to be kept in mind for a definite interpretation.

CONCLUSIONS

The radioimmunoassay for neurophysins is a substantial contribution for the study of neurohypophysial function in physiological and pathological conditions in animals and in the human. It is highly reproductible, specific and sensitive; it does not need previous blood extraction nor particular care immediately after the sampling as it is the case for ADH and oxytocin assays. It is then particularly useful for routine studies in man and preliminary results indicate that it will also be helpful for future psychopharmacological research.

However, neurophysins do not themselves share any antidiuretic or oxytocic properties; a basal level or a modification during an inhibitory test must be cautiously interpreted while stimulatory positive tests leaves little doubt. The possibility that neurophysins could be associated to other bioactive peptides than ADH and oxytocin has to be kept in mind for the final interpretation of the biological meaning of the results obtained.

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