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SPECIFIC RELEASE OF BOVINE NEUROPHYSIN II DURING ARTERIAL OR VENOUS HAEMORRHAGE IN THE COW

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SUMMARY

An almost specific release of neurophysin II with only minor release of neurophysin I was found during venous or arterial haemorrhage in five cows. Our data confirm the results of experiments *in vitro* in which vasopressin has been found to be associated with neurophysin II within neurosecretory granules.

INTRODUCTION

Neurophysins are the carriers of active neurohypophysial hormones, vasopressin (ADH) and oxytocin (Acher, Manoussos & Olivry, 1955). Since the development of specific radioimmunoassays for neurophysins and neurohypophysial hormones, many authors have been able to demonstrate a concomitant release of both neurophysins and hormones under various conditions *in vitro* and *in vivo*, thus suggesting that exocytosis plays a role in the release of active nonapeptides (see recent review by Dreifuss, 1975). Neurophysin extracted from bovine posterior pituitary glands consists of two major components designated I and II according to their electrophoretic behaviour (BN_pI and BN_pII) (Hollenberg & Hope, 1968). A third type of neurophysin, the significance of which is still unknown, has also been described (Rauch, Hollenberg & Hope, 1969) (neurophysin III). Both major types of neurophysins bind vasopressin and oxytocin *in vitro* (Breslow & Walter, 1972: Cohen, Griffin, Camier, Calzergues, Fromageot & Cohen, 1972). However, BN_pII seems to be located in neurosecretory granules containing ADH (Dean, Hope & Kazic, 1968). Conversely, the neurophysin with the higher electrophoretic mobility (N_pI) is associated with ADH in pigs (Pickup, Johnston, Nakamura, Uttenthal & Hope, 1973), rats (Burford, Jones & Pickering, 1971) and man (Legros & Louis, 1973–4).

Previously we have demonstrated a specific release of BN_pI without a release of BN_pII during milking or suckling in the cow (Legros, Reynaert & Peeters, 1974*a*). These data provided additional evidence *in vivo* in favour of exocytosis or a closely related mechanism in the physiological stimulation of the neurohypophysis. Furthermore, the results clearly showed that it was possible to elicit the specific release of one neurophysin without a release of the other one, and showed that BN_pI is associated with oxytocin in the bovine posterior pituitary.

In the present work, we have investigated the influence of haemorrhage, a stimulus which is known to induce ADH release, on the secretion of neurophysins in the cow. In a previous study (McNeilly, Legros & Forsling, 1972) haemorrhage was shown to cause release of both ADH and neurophysins in the goat. During these experiments, however, neurophysins were measured with a non-specific radioimmunoassay so that the type of neurophysin released was not defined. Studying the release of neurophysins in cows has the advantage that the bovine neurophysins can be measured with specific radioimmunoassay systems, so that a distinction can be made between bovine neurophysins I and II. In the present study we report a preferential release of BN_pII during venous or arterial haemorrhage in five cows; moreover, we give some evidence that no other immunoreactive neurophysins (e.g. neurophysin III) are released under these experimental conditions. Part of these results have been described briefly in a preliminary paper (Legros, Reynaert & Peeters, 1974*b*).

MATERIALS AND METHODS

Two cows were subjected to venous and three to arterial haemorrhage at the slaughterhouse. The day before the experiments a polyethylene catheter was inserted into the jugular vein by a method similar to that of Folley & Knaggs (1965). During all the experiments, 10 ml blood samples for the estimation of neurophysins were collected continuously from the jugular vein catheter before (control samples) and during haemorrhage. The total number of samples varied from 26 to 76 in each experiment. Total blood volume of each cow was estimated on the basis of liveweight. Haemorrhage was continued until the animals started staggering.

Blood was allowed to clot at room temperature, centrifuged and the serum kept frozen until the time of assay. The radioimmunoassay for neurophysins utilized the double antibody technique as described by Legros, Franchimont & Hendrick (1969). Antisera raised against BN_pI (A_6I) or BN_pII (A_9I) were used (Legros & Franchimont, 1974). The specificity of the radioimmunoassay system has been established by means of inhibition curve studies using various neurophysins as standards. Indirect proof of specificity was furnished by the demonstration of specific BN_pI increment, without an increase in BN_pII, during milking (Legros *et al.* 1974*a*).

In some samples we measured not only the BN_pI and BN_pII values but also the level of total immunoreactive neurophysins (BN_pT) by using the antiserum A_5IV, which contains antibodies directed against a common fragment of different neurophysins (Legros, Hendrick & Franchimont, 1970; Legros & Franchimont, 1974). In the latter system, BN_pII was used as standard and as labelled antigen.

All the samples in one experiment were measured in the same assay series, the mean intra-assay variation was $5{\cdot}68 \pm 2{\cdot}81$ (S.D.) %. Serum osmolarity was measured by means of an automatic osmometer (Advanced Instrument, Mass., U.S.A.).

EXPERIMENTAL PROCEDURES

Venous haemorrhage

Two cows with well-developed mammary veins were selected. Each vein was anaesthetized locally by s.c. injection of 10 ml 2 % procaine. After collecting control blood samples during approximately 8 min, each mammary vein was cut over a distance of 1–2 cm, the cow standing in an upright position. The outflow of blood was copious at the onset of the haemorrhage; but decreased progressively thereafter. The blood was collected continuously from both veins in marked reservoirs.

Characteristics of the individual animals were as follows.

Cow 1. Body weight 580 kg; total volume of blood which was withdrawn, 23·3 l (58·5 % of total blood volume); duration of the haemorrhage, 32 min 55 s.

Cow 2. Body weight, 600 kg; total volume of blood which was withdrawn, 18 l (42·9 % of total blood volume); duration of the haemorrhage, 46 min 40 s.

Arterial haemorrhage

Arterial haemorrhage was studied in three cows. In preparation for the later section of one carotid artery, it was brought to just beneath the skin of the neck, several weeks before the experiment, by the technique of Dhont, Houvenaghel, Peeters & Verschooten (1973).

On the day of the experiment, the transplanted carotid artery was anaesthetized by s.c. injection of 2 % procaine. After a period of control blood sampling of approximately 5 min the haemorrhage was brought about by section of the artery. The outflowing blood was, collected and the volume was measured at regular intervals. Collection of blood samples was continued until the animals started staggering, except for Cow 17 (see below).

Characteristics of the individual animals were as follows.

Cow 3. Body weight, 510 kg; total volume of blood which was withdrawn, 16·5 l (46·2% of total blood volume); duration of the haemorrhage, 25 min 45 s.

Cow 15. Body weight, 465 kg; total volume of blood which was withdrawn, 16 l (49·2% of total blood volume); duration of the haemorrhage, 11 min 45 s.

Cow 17. Body weight, 540 kg; total volume of blood withdrawn, 19 l (50·3% of total blood volume); duration of the haemorrhage, 5 min 45 s. In this case, haemorrhage was so abundant that the animal fell down 4 min after the onset. The subsequent samples had to be collected in quick succession from the carotid artery.

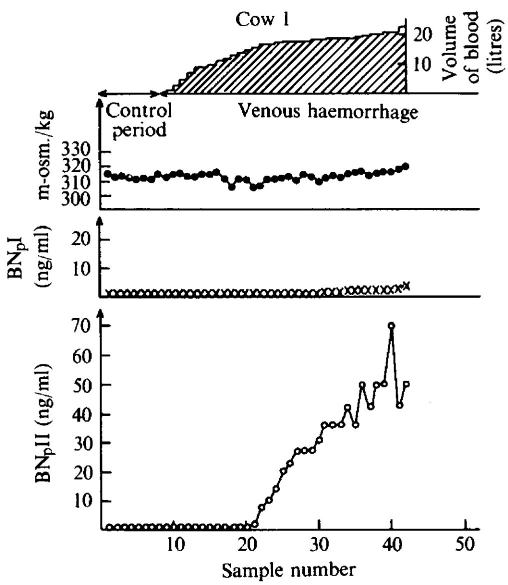

Fig. 1. Cumulative volumes of blood which were withdrawn, individual values of blood osmolarity and serum levels of bovine neurophysin I (BN_pI) and bovine neurophysin II (BN_pII) during continuous blood sampling from a jugular vein of cow 1, subjected to venous haemorrhage. Control period: 5 min 45 s; period of venous haemorrhage, 32 min 55 s.

RESULTS

Individual values of blood osmolarity, the BN_pI and BN_pII blood concentrations and the cumulative volumes of blood which were withdrawn during the venous haemorrhage in Cow 1 are shown in Fig. 1. A late increase in blood osmolarity was noted in Cow 2. A rise in the blood levels of BN_pII was observed in Cow 1 after 24% haemorrhage and in Cow 2 after 37% haemorrhage. Towards the end of the experiments, a slight increase in the blood level of BN_pI was noted in both animals. However, this increase was always considerably less ($\pm$300 %) than the rise observed for BN_pII ($\pm$3600%).

Variations in blood osmolarity and BN_pI and BN_pII blood levels, observed during arterial haemorrhage, and the cumulative volumes of blood which were withdrawn in Cow 3 are shown in Fig. 2. Only in Cow 17 was a slight increase in blood osmolarity noted. A release

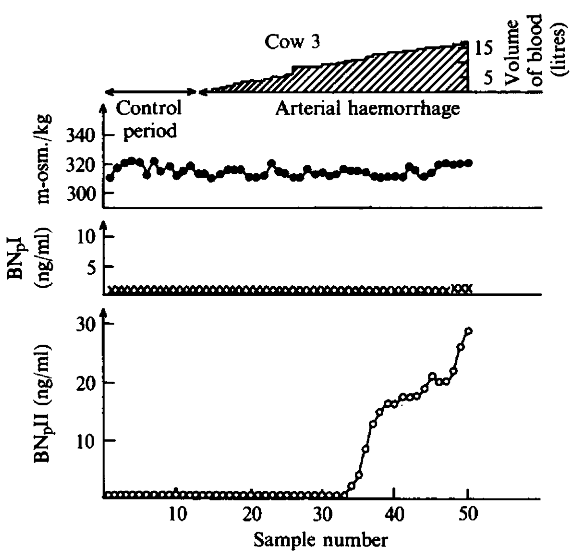

Fig. 2. Cumulative volumes of blood which were withdrawn, individual values of blood osmolarity and serum levels of bovine neurophysin I (BN_pI) and bovine neurophysin II (BN_pII) during continuous blood sampling from a jugular vein of cow 3 subjected to arterial haemorrhage. Control period, 5 min 55 s; period of arterial haemorrhage, 25 min 45 s.

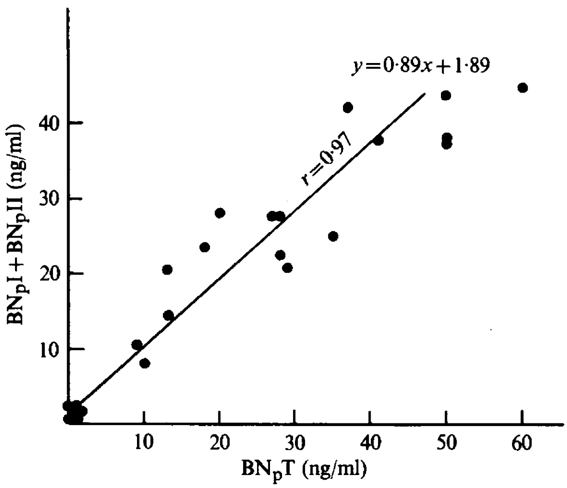

Fig. 3. Comparison between the blood levels of total immunoreactive neurophysin (BN_pT) and the sum of the blood levels of immunoreactive neurophysins I and II (BN_pI+BN_pII) in 28 blood samples from different cows subjected to haemorrhage.

of BN_pII was observed after a haemorrhage of 28% in Cow 3, of 40% in Cow 15 and of 17% in Cow 17. A very weak increase in the BN_pI level was noted in the last samples obtained from the three animals.

The comparison between the blood levels of total immunoreactive neurophysins, measured by using the antiserum A_5IV, and the sum of the blood levels of neurophysins I and II measured by using the antisera A_8I and A_9I, is shown in Fig. 3. A highly significant correlation (r = 0·97) between both values exists and the intercept of the regression curve with the abscissa is close to the origin.

DISCUSSION

The results of this work confirm earlier observations that neurophysins are released during haemorrhage (see Introduction). Moreover, we were able to demonstrate an almost specific release of neurophysin II in the cow, with only a minor increase in neurophysin I. These results are in slight disagreement with the data of Robinson, Zimmerman & Frantz (1971); these authors stated that it was almost impossible to demonstrate a specific release of BN_pII during injection of hypertonic saline. On the other hand, our data support the hypothesis of an intragranular association between neurophysin II and ADH (Dean *et al.* 1968) in cattle. Such a specificity was recently confirmed by Zimmerman, Robinson, Husain, Agosta, Frantz & Sawyer (1974) in the hypothalamus of this species.

The release of bovine neurophysin II occurs after withdrawal of a variable, but often large, volume of blood. Variations in blood osmolarity do not seem to be responsible for this increase, since the rise in the neurophysin II preceded the variations of serum osmolarity in all experiments in which an increment in serum osmolarity was observed. Hence, the neurohypophysial activation is probably related to the action of venous volo-receptors or arterial baro-receptors. In cow 17, the level of BN_pII started to rise very early, following cardiovascular collapse induced by a relatively small but very rapid haemorrhage. In this cow, activation of arterial baro-receptors very likely occurred. Such a rapid increase in the neurophysin levels has been previously reported during orthostatic fainting in men and pregnant women (Legros, Fassotte-Boxho & Demoulin, 1973). Moreover, Noble & Taylor (1953) have demonstrated in man that the release of ADH is related more to the fainting phenomenon than to the volume of blood lost during haemorrhage. Considering the effects which were observed during quick and slow haemorrhage, it may be suggested that not only the degree of haemorrhage is important but also the rate at which it occurs. A direct effect of surgical trauma itself on BN_pII release is not excluded, but is unlikely in view of the latencies which were observed in most of our experiments.

Our results, although failing to demonstrate which type of receptors are responsible for the neurohypophysial activation, confirm that cardiovascular adaptation is an important factor for the hormonal release. Further investigations are necessary to define the relationship between the cardiovascular system and neurohypophysial activity.

The good correlation which we found between the blood level of total immunoreactive neurophysins and the calculated sum of BN_pI and BN_pII levels indicates that the release of an accessory neurophysin (i.e. BN_pIII) is hardly likely. Indeed, if such a neurophysin should have been released, one would expect that the level of total immunoreactive neurophysins would be greater than the sum of BN_pI and BN_pII levels. However, a final statement cannot be made until purified BN_pIII has been tested in our immunological systems.

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