The Vasotocin-Like Biological Activity Present in the Bovine Pineal Is Due to a Compound Different from Vasotocin

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Summary

The vasotocin-like biological activity detected in an extract (E₅ fraction) of bovine pineal gland was found not to be due to the presence of vasotocin, vasopressin or oxytocin. The data obtained by means of bio- and radio-immunoassays suggest that the peptide responsible for this biological activity, however, possesses the same Pro-Arg-Gly(NH₂) tripeptidic carboxy-terminal end as vasotocin.

Introduction

In 1963, *Milcu, Pavel*, and *Neacșu* discovered in extracts of bovine pineal glands a peptide with vasopressor and oxytocic activities. Since the biological and chromatographic characteristics of this peptide were similar to those of vasotocin (AVT), the authors concluded that

this pineal peptide was "*arginine vasotocine or a hormone with similar structure*". In 1966, *Pavel* and *Petrescu* compared the antigonadotropic effects of this purified peptide with those of synthetic AVT and concluded that the peptide was identical with AVT. However, in 1972 *Neacșu*, a co-author of the 1963 paper, after a chemical analysis asserted that this peptide (termed by him E₅ fraction) was different from AVT and was composed of 14 amino-acids.

Subsequently, *Pavel* and his group, still using the bioassay approach, concluded: that the pineal gland of all mammals so far examined, including man, contained AVT (or lysin vasotocin, in pigs), that AVT was synthesized by the ependymal cells in the pineal recess and by the cells of the subcommissural organ (SCO), and that AVT was released into the CSF (for references, see *Pavel*, 1978). These conclusions were largely supported by the radioimmunoassay detection of AVT in the pineal of different mammalian species (*Rosenbloom* and *Fisher*, 1975 a; *Fernstrom et al.*, 1980) and in the rabbit SCO (*Rosenbloom* and *Fisher*, 1975 b), the immunocytochemical detection of AVT in the rat pineal (*Bowie* and *Herbert*, 1976), and especially by the chemical characterization—by means of amino-acid analysis and mass spectrometry—of AVT in bovine pineal gland (*Cheesman*, 1970). As, moreover, exogenously administered AVT appears to be inhibitory to many aspects of reproduction (for review see *Vaughan* and *Blask*, 1978), it is easy to understand why, despite of the fact that the presence of AVT in the pineal was always contested (*Ebels et al.*, 1965), most of the authors have completely forgotten the conclusion raised by *Neacșu* (1972).

Recently, using a specific radioimmunoassay for AVT, *Dogterom et al.* (1979, 1980) and *Negro-Vilar et al.* (1980) were unable to demonstrate AVT immunoreactivity in the mammalian pineal gland. It was also observed that the AVT immunoreactivity obtained by means of immunocytochemistry in rat pineal was due to an immunoreaction of the antiserum with an unidentified compound (*Pévet et al.*, 1980 a). Moreover, it has been demonstrated that the neurophysins observed in the pineal—assumed to indicate the presence of AVT (see *Pavel*, 1978)—were only the carrier proteins of vasopressin (AVP) and oxytocin (OT) (*Pévet et al.*, 1980 b). Their presence (*Dogterom et al.*, 1979, 1980) was explained by the occurrence in the pineal of fibres originating from the hypothalamic magnocellular nuclei (*Buijs* and *Pévet*, 1980). However, using another AVT-bioassay than that of *Pavel*'s group, we were able to find an AVT-biological activity in the pineal of several mammals (*Holder et al.*, 1981; *Pévet et al.*, 1981). This made it necessary to reconsider *Neacșu*'s conclusion (1972) that the AVT-biological activity in the mammalian pineal was due

to a compound different from AVT itself. We have therefore tested the biological and immunological activities of the E₅ bovine pineal fraction, prepared by Dr. Neacșu according to the original procedure (Milcu *et al.*, 1963).

Materials and Methods

After preparation by Dr. Neacșu, the E₅ fraction was sent to Amsterdam by airmail. For comparison, bovine pineals obtained from Pel-Freez Biological Inc., Rogers, Arkansas, U.S.A., were used. They arrived in solid CO₂ and were stored at -70 °C until used. All experiments reported in the present paper have been done independently in different laboratories by different teams, none of which had any knowledge of the results obtained by the others.

Radioimmunoassays for AVT, AVP and OT (radioimmunoassays—Amsterdam). The three radioimmunoassays which discriminate between all three hormones and measure them in the same sample, were developed in the Netherlands Institute for Brain Research, Amsterdam. The procedure used in our laboratory for raising antibodies, the preparation and purification of labelled hormones, the preparation of the standard curve and the extraction of the hormones from the sample by means of activated vycor glass powder have been described earlier (Dogterom *et al.*, 1977, 1980).

Radioimmunoassay for AVT/AVP (radioimmunoassay—Geneva). In the radioimmunoassay for AVP/AVT, developed by the Division of Endocrinology of the Cantonal Hospital in Geneva, an antibody against the last three amino-acids of AVP or AVT, *i.e.*, Pro-Arg-Gly(NH₂), was used. The procedure and the characteristics of this RIA have been published previously (Czernichow *et al.*, 1974).

Bioassay (Strasbourg). A new and very sensitive bioassay for AVT was used which is based on the capacity of AVT to induce, *in vitro*, isometric contractions in eel ventral aorta strip mounted in a small perspex micro-chamber. This bioassay has been described earlier (Holder and Guerné, 1976; Holder *et al.*, 1981).

Results and Discussion

The results obtained with the E₅ bovine pineal fraction are represented in Table 1. With the specific and sensitive RIAs developed in Amsterdam no immunoreactivity for AVT, AVP or OT could be detected in this fraction. This confirms the findings of Milcu *et al.* (1963) that the vasopressor and oxytocic activities observed in this fraction were not due to AVP or OT. It also confirms the conclusion of Neacșu (1972) that AVT was not present in the fraction. However, our findings obtained with the bioassay demonstrated the presence of an AVT-like biological activity in the E₅ fraction and thus confirmed again the work of Milcu *et al.* (1963).

Table 1. Results obtained in the E_5 fraction with the different assays

Biological test		Radioimmunoassay—Amsterdam		Radioimmunoassay—Geneva
AVT-biological activity		AVP	OT	AVP/AVT/X-Pro-Arg-Gly(NH ₂) in
1000 pg E_5	*	38 pg equivalent AVT	undetectable	** 45 pg equivalent AVP
		37 pg equivalent AVT		** 50 pg equivalent AVP
	*	36 pg equivalent AVT		
		47 pg equivalent AVT		
	*	31 pg equivalent AVT		
		23 pg equivalent AVT		
		28 pg equivalent AVT		

* Results obtained with different dilutions in the same assay.

** Triplicate.

Table 2. Neurohypophysial hormones and AVT-biological activity in bovine pineal

Bioassay	Radioimmunoassays	
AVT-biological activity	AVP	AVT OT
1.7 pg equivalent AVT/pineal	2.80 $\pm$ 0.59 pg/mg wet tissue (between 250 and 800 pg/gland)	undetectable
(9)	(16)	(16)
		3.90 $\pm$ 2.14 (between 250 and 800 pg/gland)

(x) Number of assays.

The RIA for AVT/AVP developed in Geneva made use of an antibody against the tripeptidic carboxyterminal end of AVP or AVT, *i.e.*, Pro-Arg-Gly(NH₂). This antibody is probably also able to react with other compounds ending by Pro-Arg-Gly(NH₂) as suggested by the fact that it could react with the synthetic tripeptide Pro-Arg-Gly(NH₂) (Czernichow *et al.*, 1974). With this RIA we have found an immunoreactivity in the E₅ fraction the dilution curve of which was exactly parallel to the standard (here AVP) (Fig. 1). Moreover, after incubation of E₅ with trypsin, the antibody did not longer bind this peptide. This implies that the peptide present in this fraction is most probably terminating by Pro-Arg-Gly(NH₂), a result in accordance with Neacşu's observation (1971) unfortunately published only in abstract form. An AVT-biological activity is also found in the bovine pineal gland (*cf.* Table 2). This AVT-biological activity, however, is very low. In view of the properties of the bioassay especially *vis-à-vis* of AVP and OT (Holder *et al.*, 1981), this low AVT-biological activity cannot be attributed to the presence of AVP or OT. It appears therefore that a compound different from AVT but with a biological activity similar to that of AVT is also detectable in the intact bovine pineal. As a consequence it is possible to assert that the results obtained with the E₅ fraction are not due to the procedure used to isolate the fraction.

Fig. 1. Representative standard curve of AVP with Pro-Arg-Gly(NH₂)-antiserum and cross reaction curve with the E₅ fraction of Neacşu

As synthetic AVT is being used as reference in the bioassay (Holder and Guerné, 1976), the results are expressed in equivalents of synthetic AVT. It is evident that the value found does not correspond with the real amount which, the peptide being not identified, cannot be determined.

From these different results we conclude that in the bovine pineal a compound is present, which differs from AVT but probably ends, as AVT, by Pro-Arg-Gly(NH₂) and is responsible of the AVT-biological activity. This conclusion is strengthened by the fact that, applying two non-related techniques, the bioassay used and the Geneva RIA, approximately the same values were obtained (Table 1).

These results contradict the conclusion of Pavel and his group that AVT is a pineal hormone. However, this contradiction *may only be apparent*. On the one hand, by denying the presence of AVT in the bovine pineal as well as in the pineal of other mammals (Dogterom et al., 1979, 1980; Pévet et al., 1979, 1980 a) we contest the conclusion of Pavel (see details in Pavel, 1978). On the other hand, however, our bioassay findings in the bovine pineal as well as in that of many other species (Holder et al., 1981; Pévet et al., 1981), confirm the experimental basis of Pavel's work.

In connection with the present findings it is of interest to recall that the E₅ bovine pineal fraction blocks spermatogenesis in gonadotropin-treated male frogs (Neacșu, 1972) and inhibits uterine weights and P³² incorporation in gonadotropin-treated female mice (Neacșu, 1972). Moreover the E₅ fraction acts on pituitary levels of LH and prolactin in intact rats (Vaughan et al., 1980) and is very active in the compensatory ovarian hypertrophy test (unpublished results). The peptide in the E₅ fraction which has some characteristics in common with AVT might therefore represent the—or one of the—pineal peptidic antigonadotropic hormone(s).

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