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Testicular dysfunction in experimental chronic renal insufficiency: a deficiency of nocturnal pineal *N*-acetyltransferase activity

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Summary. Biochemical correlates of neuroendocrine/gonadal function and nocturnal levels of serotonin *N*-acetyltransferase (NAT) activity were determined in partially nephrectomized (PNx), male, Long Evans rats following a 5-week period of chronic renal insufficiency (CRI). PNx animals demonstrated two to four-fold elevations in urea nitrogen and three to four-fold reductions ($P<0.02$) in plasma total testosterone concentrations as compared to sham-operated controls. The pituitary LH contents of PNx rats were decreased to approximately 60% of the control value ($P<0.05$). There were no differences in plasma prolactin levels between the control and PNx groups either at mid-day or in the middle of the night. Nocturnal pineal NAT activity in PNx rats was markedly reduced to approximately 20% of the control value ($P<0.001$). Similar evidence of gonadal dysfunction (reduced plasma total testosterone and testes testosterone content) and a significant decrease in night-time levels of pineal NAT activity were also observed after 13 weeks of CRI in PNx rats of the Sprague–Dawley strain that were housed under a different photoperiod. These results suggest that pineal gland dysfunction is a feature of CRI in the PNx model. Such an abnormality might contribute to the pathogenesis of gonadal dysfunction in CRI.

Keywords: pineal gland, serotonin *N*-acetyltransferase, subtotal nephrectomy, hypothalamic–gonadal axis, testosterone, prolactin, luteinizing hormone

Functional abnormalities at various levels of the hypothalamic–pituitary–testicular axis are commonly observed in humans and experimental animals with chronic renal insufficiency (CRI) (Cowden *et al.* 1981; Lim & Fang 1975). Manifestations of these abnormalities include varying degrees of spermatogenic arrest (Holdsworth *et al.* 1977; Aumiller *et al.* 1981) decreased serum testosterone concentrations (Blackman *et al.* 1981; Briefel *et al.* 1982) and abnormalities in plasma concentrations of gonadotrophins and prolactin (Emmanouel *et al.* 1980). The pathophysiology of gonadal dysfunction in uraemia is extremely complex as it appears to be caused by a variety of factors including impaired testicular steroidogenesis (Briefel *et al.* 1982), decreased clearance of pituitary hormones (Emmanouel *et al.* 1980), and secretory defects at the levels of the hypothalamus and the pituitary (Schalch *et al.* 1975; Cowden *et al.* 1981).

The pineal gland represents an additional level of regulation within the sexual axis of

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mammals (Mess *et al.* 1979; Reiter 1980). For example melatonin, one of the major indoleamines secreted by the pineal, has been shown to influence gonadal function at the levels of the midbrain, the hypothalamus, the anterior pituitary and, possibly, the testes (Mess *et al.* 1979; Demaine & Kann 1981; Martin *et al.* 1982; Jarrige *et al.* 1984). In addition, there is considerable evidence that the pineal gland secretes other indoleamines and peptides which may, likewise, modulate function at various levels of the sexual axis (Pevet 1983*a*; *b*). Many aspects of pineal function, including the activity patterns of hormone biosynthesis and secretion, demonstrate circadian rhythms. These rhythms are regulated primarily by postganglionic sympathetic neural projections from the superior cervical ganglia (Ariens Kappers 1965). One of the best characterized of these circadian rhythms is that of serotonin *N*-acetyltransferase (E.C.2.3.1.5) (NAT), which catalyses a key step in the biosynthesis of melatonin. NAT activity increases during the dark phase of the light/dark cycle to levels as much as fifty-fold greater than those observed during the light phase (Binkley 1983). The pineal NAT rhythm is regulated by beta and alpha$_1$ adrenoceptors, with cyclic AMP serving as an intracellular second messenger (Sugden & Klein 1985). Circadian changes in pineal and plasma levels of melatonin are closely coupled to the NAT rhythm (Welker & Vollrath 1984). NAT activity is considered to be a biochemical marker for the activation of the pineal gland by adrenergic mechanisms (Lewy 1983).

Several lines of evidence suggest the possibility of abnormal pineal function in CRI. First, many of the neuroendocrine abnormalities observed in patients and animal models of CRI involve mechanisms that are known to be influenced by the pineal gland in intact mammals. Furthermore, it is possible that the generalized impairment of adrenergic function that has been previously observed in uraemia (Souchet *et al.* 1986) might affect the cyclic activity of the gland. Finally, previous studies indicate that pineal function is subject to feedback regulation by pituitary and gonadal hormones (Rudeen & Reiter 1980). For example, castrated male rats demonstrate an abnormal pineal serotonin content (Vacas & Cardinali 1979), a decreased pineal cyclic AMP content (Karasek *et al.* 1978), and a reduction in nocturnal pineal NAT activity (Rudeen & Reiter 1980). Therefore, abnormalities in the plasma levels of reproductive hormones in CRI could affect pineal function. The present study was undertaken to investigate pineal function in the partially nephrectomized (PNx) rat model of CRI. For this purpose, several indices of pituitary/gonadal function were evaluated together with the nocturnal pineal NAT activity, which was used as a marker for pineal activation by adrenergic mechanisms.

Materials and methods

Animal surgery and care

Chronic renal insufficiency was induced in male rats by sub-total nephrectomy using a two-stage surgical procedure as described previously (Holmes & Kahn 1987). Control animals received sham operations in which each kidney was exposed and manipulated. In Experiments I and II, Long Evans rats (65–79 days of age; 290–350 g) were housed under a 14:10 light: dark cycle and studied 5 weeks after the induction of CRI. In Experiment III, CRI was induced in 35-day-old (125 g) Sprague–Dawley rats (Harlan, Indianapolis, IN) which were housed under a 12:12 light:dark cycle and studied after 13 weeks. In all cases, animals were maintained at 23 ± 1°C and received food (Wayne Lab Blox MRH 22/5 Allied Mills, Inc., Chicago, IL) and water *ad libitum*.

Specimen collection

Heparinized blood was collected from the tail at mid-day (6–7 h after the onset of the light period) or from the trunks of animals killed by decapitation at night (4 to 5 h after the

onset of the dark period). In the latter case, animals were exposed briefly to dim red light prior to sacrifice. Tissues (pituitary gland, pineal gland and testis) were rapidly removed, frozen on dry ice, and stored at −70°C prior to assay. Plasma prepared from whole blood by centrifugation was similarly stored prior to analysis.

Hormone assays

Plasma testosterone was determined using a commercial kit (Immuno-Direct, Pantex, Santa Monica, CA). Dihydrotestosterone cross-reactivity was 7% relative to testosterone in this assay. Testosterone assays were carried out on tissue extracts using the same assay kit. In this case, 100 mg (wet weight) of testis was homogenized in 1 ml dichloromethane. After centrifugation, the organic phase was evaporated to dryness, reconstituted on 1.25 ml charcoal-stripped human serum and assayed by the usual procedure. The recovery of tritiated testosterone from tissue extracts prepared in this fashion was 63±2% (mean±s.d., $n=4$). Free testosterone was determined by ultrafiltration as described by Vlahos *et al.* (1982). Pituitary LH and plasma prolactin were assayed by RIA using reagents and protocols prepared by Dr A. F. Parlow and supplied by the National Hormone and Pituitary Program of the NIADDK. LH and prolactin are expressed relative to the rLH-RP-2 and rPRL-RP-3 reference preparations, respectively. Pituitary glands were homogenized in 0.5 ml of 0.05M phosphate buffer, pH 7.0 and the homogenate was diluted one thousand-fold prior to assay.

N-Acetyltransferase assay

Pineal NAT activity was determined by the method of Namboodiri *et al.* (1980) using glands that were collected at night. Briefly, each gland was placed in a microconical polyethylene tube along with 20 µl of 0.25 M ice-cold sucrose and homogenized for 10 s using a Microultrasonic Cell Disrupter (Kontes). After centrifugation at 10 000 *g* for 2 min, 5 µl of supernatant was added to 200 µl of a buffer–substrate mixture containing 100 mM Na phosphate, pH 6.8; 10 mM tryptamine HCl, 0.5 mM acetyl CoA and 120 000 dpm [acetyl-1-^{14}C] acetyl CoA. Each reaction mixture was incubated at 37°C for 15 min and stopped by the addition of 1 ml chloroform. After shaking, the chloroform extract was washed as described by Namboodiri *et al.* (1980) and the NAT reaction product, ^{14}C-*N*-acetyltryptamine in the organic phase, was quantitated by liquid scintillation counting. NAT activity is expressed in terms of nmol product formed/h/pineal gland.

Other assay and statistical procedures

BUN was determined using the Astra 4 Analyzer (Beckman Instruments, Brea, CA). The significance of differences between group means was evaluated using Student's *t*-test for unpaired samples.

Results

Details of the three experiments and some of the characteristics of control and PNx animals after the induction of CRI are shown in Table 1. The percentages of the total renal mass removed during the partial nephrectomy in Experiments I–III were (mean±s.d.) 72±2, 75±4 and 65±3%, respectively. PNx animals demonstrated significant increases in plasma urea nitrogen as compared to controls with values ranging from 14 to 28, 22 to 35 and 14 to 28 mmol/l in Experiments I–III, respectively. The magnitudes of these elevations are consistent with a state of moderate to severe CRI based on previously established criteria (Ormrod & Miller 1980). The body weights of PNx animals increased at a slower rate than those of the controls. There were no significant differences in testis weights between control and PNx groups; however, the relative testis weights of the PNx groups were typically increased.

Table 1. Details of the experiments and some characteristics of control and partially nephrectomized rats at necroscopy

Exp.	Strain*	Photo-period L:D	Duration of uraemia (weeks)	Group (*n*)	Body Wt. (g)	Urea nitrogen (mmol/l)	Testis weight absolute (g)	Testis weight relative (mg/100g)
I	LE	14:10	5	Control (6)	395±6	7.1±0.1	3.07±0.13	784±29
				PNx (7)	367±9*	16.6±1.8**	3.37±0.07	913±27**
II	LE	14:10	5	Control (6)	444±17	8.2±2.9	3.31±0.09	753±35
				PNx (12)	370±11*	26.1±1.2**	3.01±0.17	812±32
III	SD	12:12	13	Control (6)	371±12	7.5±0.2	3.79±0.07	931±32
				PNx (6)	336±13	19.6±1.8***	3.49±0.12	1046±39*

Male rats were partially nephrectomized (PNx) or sham operated as described in Methods. Measurements of body and testis weights and urea nitrogen were carried out after either 5 or 13 weeks of renal insufficiency. Relative testes weight is expressed in mg/100 g body weight. LE, Long Evans; SD, Sprague–Dawley. Asterisks denote level of significance of differences between the means of the control and PNx groups. * $P<0.05$; ** $P<0.01$. *** $P<0.001$ as evaluated using Student's *t*-test (two tailed) for unpaired samples.

Values for the various biochemical indices of neuroendocrine/gonadal function are summarized in Table 2. Plasma testosterone concentrations in PNx rats were reduced relative to the control values in all three experiments. Plasma testosterone was decreased at mid-day in Experiment II. However, the night-time difference between the control and PNx group was not statistically significant. This may be due to the fact that three of the PNx animals had extremely high values relative to the rest of the group. Since rats with CRI are known to develop hypoproteinaemia as well as alterations in the degree of protein binding in plasma, the validity of decreased plasma testosterone as a marker for reduced testicular function was further examined by measuring free testosterone and the testosterone content of testis tissue. The fraction of testosterone in the free form was slightly increased in PNx animals of Experiment II as compared to controls; however, free testosterone concentrations in these PNx rats paralleled the values for total testosterone (i.e., they were reduced). Testis testosterone levels in PNx animals of Experiment III were reduced to approximately 50% of the control value after 13 weeks of CRI, supporting the notion that the observed decrease in plasma testosterone was due to a decrease in testicular steroidogenesis.

Plasma prolactin concentrations at night were similar in the control and PNx groups in Experiments I and II. In a separate investigation of Long Evans males which were prepared and housed under identical conditions, plasma prolactin concentrations at mid-day in the control and PNx groups were (mean±s.e., $n=5$) 13.6±1.8 and 14.2±2.0 ng/ml, respectively. Pituitary LH levels in the PNx groups demonstrated consistent decreases to 60 and 66% of the control values in Experiments I and II, respectively.

Night-time pineal NAT activities in the sham-operated controls were in excellent agreement with those previously reported for intact rats housed under the same photoperiod (Rudeen & Reiter 1980). In contrast, PNx animals in all three experiments demonstrated significantly decreased levels of pineal NAT activity at night. NAT activities in the PNx animals of Experiments I and II averaged only 20% of those of sham-oper-

Table 2. Biochemical indices of neuroendocrine/gonadal function in control and partially nephrectomized rats

Parameter (units)	Exp.	Group: Control mean ± s.e.(*n*)	Group: PNx mean ± s.e.(*n*)	*P* value
Plasma total Testosterone (mg/dl)	I	3.5±0·6(6)	1.0±0.6(7)	<0.02
	II (AM)	1.7±0.3(8)	0.4±0.1(10)	<0.001
	II	2.1±0.5(8)	2.0±0.6(12)	<0.9
	III	2.3±0·6(6)	0.4±0.1(6)	<0.02
Plasma free testosterone (%)	II	6.0±0.2(4)	7.2±0.5(6)	<0.07
Plasma free testosterone (pg/ml)	II	162±52(4)	67±47(5)	<0.2
Testis testosterone (ng/g wet wt)	III	102±15(6)	46±15(6)	<0.05
Plasma prolactin (ng/ml)	I	8.5±1.6(4)	7.9±0.9(6)	<0.80
	II	6.8±0.9(4)	7.6±0.6(8)	<0.49
Pituitary luteinizing hormone (μg/gland)	I	2.1±0.3(6)	1.3±0.1(6)	<0.035
	II	2.4±0.5(8)	1.6±0.1(11)	<0.08

Long Evans rats housed under a 14:10 (light:dark) photoperiod were studied after 5 weeks of CRI in Exps I and II. Sprague–Dawley rats housed under a 12:12 photoperiod were studied after 13 weeks of CRI in Exp. III. Biochemical indices were determined as described in Methods. Assays were performed on plasma or tissues obtained 6–7 h after the onset of the dark period with the exception that plasma testosterone in Exp. II was also performed at mid-day (AM). The level of significance of differences between the means of the control and PNx groups were calculated using Student's *t*-test for unpaired samples. Two-tailed *P* values are presented.

ated controls (Fig. 1). After 13 weeks of CRI, Sprague–Dawley rats housed under a 12:12 (light:dark) photoperiod had a night-time pineal NAT activity of 58±11% of that observed in sham-operated controls ($P<0.01$).

Discussion

PNx animals with moderate to severe CRI of 5 or more weeks duration showed evidence of testicular, pituitary and pineal dysfunction. The decrease in plasma total and free testosterone and the reduced testis testosterone content we observed are consistent with decreased testicular steroidogenesis in the PNx model. The decrease in pituitary LH content further suggests either a reduction in LH synthesis or an increase in LH secretion by the pituitary gonadotrophs. Since previous studies have shown that *plasma* LH concentrations are markedly decreased in chronically uraemic PNx rats as compared to sham-operated controls (Briefel *et al.* 1982; Handelsman & Turtle 1981), the decreased pituitary LH observed in the present study probably reflects a decrease in pituitary gonadotrophin output by PNx males.

It has been shown that reduced testicular steroidogenesis in the PNx rat is due, in part, to a defect at the level of the testis, distal to the cyclic AMP production step (Briefel *et al.* 1982). One additional factor that has been proposed to contribute to gonadal dysfunc-

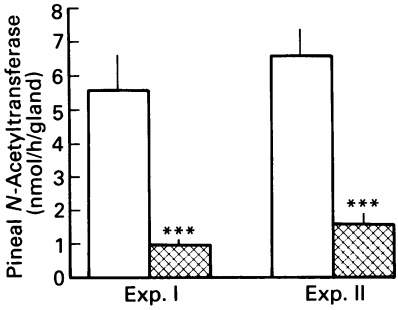

Fig. 1. Nocturnal *N*-acetyltransferase activity in pineal homogenates from partially nephrectomized and sham-operated control rats. Supernatants (10 000 *g*; 2 min) were prepared from pineal glands collected at night and assayed for NAT activity as described in Methods. □, Control; ▩, PNx groups. The error bar denotes one s.e. Asterisks indicate the significance level of the differences between the means, *** $P<0.001$, *t*-test for unpaired specimens (two tailed).

tion in the PNx model is the suppression of the pituitary by hyperprolactinaemia (Briefel *et al.* 1982). Our observation that plasma prolactin concentrations in PNx rats were similar to those of controls, both at mid-day and at night, suggest that hyperprolactinaemia does not contribute to gonadal dysfunction under the conditions of the present study.

In a previous study of gonadal function in male rats made nephrotic by purine aminonucleoside administration, nephrotic (but non-uraemic) rats demonstrated decreased plasma levels of testosterone and LH as well as decreased pituitary LH contents (Glass *et al.* 1985). The hypogonadotrophic hypogonadism in this nephrotic model was attributed to an increased sensitivity of the pituitary gonadotrophs to negative feedback by testosterone (Glass *et al.* 1985). The authors suggested that hypoalbuminaemia (or some other factor associated with the nephrotic state) might affect gonadal/pituitary feedback mechanisms by altering the bioavailability of various circulating androgen fractions. Since proteinuria and hypoalbuminaemia are also present in the PNx model (Shimamura & Morrison 1975) as early as 6 weeks post-nephrectomy (Holmes & Kahn 1987), hypogonadotrophic hypogonadism in both models might occur by a common mechanism.

Our studies demonstrate that CRI in the PNx model is accompanied by changes in the phase or amplitude of the circadian rhythm of pineal NAT activity. This finding implies a generalized abnormality in the cyclic activity of the gland with the possibility of changes in the diurnal rhythm of the synthesis and secretion of melatonin, other pineal indoleamines, and pineal peptides. It is unlikely that the deficiency of nocturnal pineal NAT activity in PNx animals was due to stress, as the pineal NAT rhythm of rodents has been shown to be refractory to both short-term (Welker & Vollrath 1984) and long-term (Vaughan *et al.* 1985) stress-related activators of the sympathetic nervous system.

The abnormal nocturnal NAT activity in PNx animals may be a manifestation of a generalized impairment of adrenergic function in CRI. Previous studies have shown that the uraemic state is characterized by a decreased beta adrenoceptor-mediated responsiveness (Souchet *et al.* 1986); the presence of alpha$_1$ receptors with altered binding properties (Meggs *et al.* 1986); and abnormal regulation of the membrane adenylate cyclase (Jacobsson *et al.* 1985). One or more of these factors might similarly affect adrenergic function in the pineal and thus account for the decreased nocturnal NAT activity that we observed.

Finally, abnormal pineal function in PNx rats could be secondary to the marked impairment of testicular function in this model of CRI. A number of investigators have demonstrated effects of gonadal and pituitary hormones on pineal morphology and biosynthetic activity including the various castration-induced changes mentioned in the introduction. The more subtle abnormalities in testicular function that accompany CRI might similarly affect pineal biosynthetic mechanisms by altering some aspect of gonadal–pineal feedback regulatory mechanisms.

In conclusion, we have found that male rats with moderate to severe CRI demonstrate pathological changes within the sexual axis that indicate a state of hypogonadotrophic hypogonadism. The circadian rhythm of pineal NAT activity in PNx animals also appears to be abnormal. This abnormality may be secondary to an impairment in adrenergic function or to a decrease in testicular function. However, since experimental manipulation of the pineal melatonin rhythm in intact rodents has been shown to modulate neuroendocrine/gonadal function at various levels of the sexual axis (Demaine & Kann 1979; Martin *et al.* 1982; Ronnekleiv *et al.* 1973), a functional defect at the level of the pineal gland might contribute as a primary factor to the pathophysiology of gonadal dysfunction in the PNx model.

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References

Ariens Kappers J. (1965) Survey of the innervation of the epiphysis cerebri and the accessory pineal organs in vertebrates. *Progr. Brain Res.* **10**, 87–151.

Aumiller G., Ritz E., Kreusser E. & Spiegelberg U. (1981) Ultrastructure of the rat testes in experimental uremia. *Andrologia* **13**, 163–173.

Binkley S.A. (1983) Circadian rhythms of pineal function in rats. *Endo. Rev.* **4**, 255–270.

Blackman M.R., Weintraub B.D., Kourides I.A., Solano J.T., Santer T. & Rosen S.W. (1981) Discordant elevation of the common alpha subunit of the glycoprotein hormones compared to beta subunits in serum of uremic patients. *J Clin. Endocrinol. Metab.* **53**, 39–48.

Briefel G.R., Panayiotis D., Tsitouras D., Kowatch M.A., Harman S.M. & Blackman M.R. (1982) Decreased *in vitro* testosterone production by isolated Leydig cells from uremic rats. *Endocrinology* **110**, 976–981.

Cowden, E.A., Ratcliffe W.A., Ratcliffe J.G. & Kennedy A.C. (1981) Hypothalamic–pituitary function in uremia. *Acta Endocrinol.* **98**, 488–495.

Demaine C. & Kann H.C. (1979) Modifications of the electrical activity of hypothalamic neurons by pineal indoles. *Progr. Brain Res.* **52**, 373–375.

Ellis G. & Desjardins C. (1982) Male rats secrete luteinizing hormone and testosterone episodically. *Endocrinology* **110**, 1618–1627.

Emmanouel D.S., Lindheimer M.D. & Katz A.I. (1980) Pathogenesis of endocrine abnormalities in uremia. *Endocrinol. Rev.* **1**, 28–44.

Glass A.R., Beach J. & Vigersky R.A. (1985) Hypogonadotropic hypogonadism in nephrotic rats: increased sensitivity to negative feedback effects of testosterone. *Metabolism* **34**, 574–579.

Handelsman D.J. & Turtle J.R. (1981) Experimental uremic hypogonadism in the male rat. *Proceedings of the 63rd Annual Meeting of the Endocrine Society*, Cincinnati, OH. p. 97 (Abstract 57).

Holdsworth S., Atkins R.C. & DeKretser D.M. (1977) The pituitary–testicular axis in men with chronic renal failure. *N. Engl. J. Med.* **296**, 1245–1249.

Holmes E.W. & Kahn S.E. (1987) Tryptophan distribution and metabolism in experimental chronic renal insufficiency. *Exp. Mol. Pathol.* **46**, 89–101.

Jacobsson B., Ransnas L., Nyberg G., Bergh C.H., Magnusson Y. & Hjalmarson, A. (1985) Abnormality of adenylate cyclase regulation in human platelet membranes in renal insufficiency. *Eur. J. Clin. Invest.* **15**, 75–81.

Jarrige J.F., Thieblot P. & Boucher D. (1984) Lack of melatonin action on testosterone production by superfused rat interstitial cells. *Acta Endocrinol.* **107**, 117–124.

Karasek M., Karasek E. & Stepien H. (1978) Effect of castration on the concentration of adenosine-3′, 5′-monophosphate in the rat pineal organ. *J. Neural Transm.* **42**, 145–150.

Lewy A.J. (1983) Melatonin secretion—a marker for human pineal function. In *The Pineal Gland and its Endocrine Role*. Eds. J. Axelrod, F. Fraschini & G.P. Velo. New York: Plenum. pp. 521–533.

Lim V.S. & Fang V.S. (1975) Gonadal dysfunction in uremic men. *Am. J. Med.* **58**, 655–662.

Martin J.E., McKeel D.W. & Sattler C. (1982) Melatonin directly inhibits gonadotroph cells. *Endocrinology* **110**, 1079–1084.

Meggs L.G., Ben-Ari J., Gammon D., Choudhury M. & Goodman A.I. (1986) Effect of chronic uremia on the cardiovascular alpha$_1$ receptor. *Life. Sci.* **39**, 169–179.

Mess B., Trentini G.P., Ruzsas C. & DeGaetani

C.F. (1979) Some endocrine effects of the pineal gland and melatonin with special reference to reproduction. *Prog. Brain Res.* **52**, 329–340.

NAMBOODIRI M.A.A., WELLER J.L. & KLEIN D. (1980) Evidence of inactivation of pineal indoleamine *N*-acetyltransferase by protein thiol: disulfide exchange. *J. Biol. Chem.* **255**, 6032–6035.

ORMROD D. & MILLER T. (1980) Experimental uremia: description of a model producing varying degrees of stable uremia. *Nephron* **26**, 249–254.

PEVET P. (1983*a*) The 5-methoxyindoles different from melatonin: their effects on the sexual axis. In *The Pineal Gland and its Endocrine Role.* Eds J. Axelrod, F. Fraschini & G.P. Velo. New York: Plenum. pp. 113–149.

PEVET P. (1983*b*) The different classes of proteic and peptidic substances present in the pineal gland. In *The Pineal Gland and its Endocrine Role.* Eds J. Axelrod, F. Fraschini & G.P. Velo. New York: Plenum. pp. 331–348.

REITER R.J. (1980) The pineal and its hormones in the control of reproduction in mammals. *Endocrinol. Rev.* **1**, 109–131.

RONNEKLEIV O.K., KRULICH L. & MCCANN S.M. (1973) An early morning surge of prolactin and its abolition by pinealectomy. *Endocrinology* **92**, 1339–1342.

RUDEEN P.K. & REITER R.J. (1980) Depression of nocturnal pineal serotonin *N*-acetyltransferase in castrate male rats. *J. Neural Transm.* **48**, 1–8.

SCHALCH D.S., GONZALEZ-BARCENA D., KASTIN A.J., LANDA L., LEE L.A., ZAMORA M.T. & SCHALLY A.V. (1975) Plasma gonadotropins after administration of LH-releasing hormone in patients with renal or hepatic failure. *J. Clin. Endocrinol. Metab.* **41**, 921–925.

SHIMAMURA T. & MORRISON A.B. (1975) A progressive glomerulosclerosis occurring in partial five-sixth nephrectomized rats. *Am. J. Pathol.* **79**, 96–106.

SOUCHET T., BREE F., BAATARD R., FONTENAILLE C., D'ATHIS P., TILLEMENT J.P., KIECHEL J.R. & LHOSTE F. (1986) Impaired regulation of beta$_2$-adrenergic receptor density in mononuclear cells during chronic renal failure. *Biochem. Pharmacol.* **35**, 2513–2519.

SUGDEN D. & KLEIN D.C. (1985) Regulation of rat pineal α_1-adrenoceptors. *J. Neurochem.* **44**, 63–67.

VACAS M.I. & CARDINALI D.P. (1979) Effects of castration and reproductive hormones on pineal serotonin metabolism in rats. *Neuroendocrinology* **28**, 187–195.

VAUGHAN G.M., TAYLOR T.J., PRUITT B.A. & MASON A.D. (1985) Pineal function in burns: melatonin is not a marker for general sympathetic activity. *J. Pineal Res.* **2**, 1–12.

VLAHOS I., MACMAHON W., SGOUTAS D., BOWERS W., THOMPSON J. & TRAWICK W. (1982) An improved ultrafiltration method for determining free testosterone in serum. *Clin. Chem.* **28**, 2286–2291.

WELKER H.A. & VOLLRATH (1984) The effects of a number of short-term exogenous stimuli on pineal serotonin-*N*-acetyltransferase activity in rats. *J. Neural Transm.* **59**, 69–80.