Aust. J. Biol. Sci., 1988, **41**, 393–400

Structure–Activity Studies of Melatonin Analogues in Prepubertal Male Rats

D. J. Kennaway,[A] *H. M. Hugel,*[B] *S. Clarke,*[B] *A. Tjandra,*[B] *D. W. Johnson,*[C] *P. Royles,*[A] *H. A. Webb*[A] *and F. Carbone*[A]

[A] Department of Obstetrics and Gynaecology, University of Adelaide, S.A. 5000.
[B] Department of Applied Chemistry, Phillip Institute of Technology, Plenty Road, Bundoora, Vic. 3083.
[C] Department of Chemical Pathology, Adelaide Children's Hospital, King William Road, North Adelaide, S.A. 5006.

Abstract

Comparison has been made between the activity of the pineal hormone melatonin, and several analogues and metabolites in inhibiting sexual development in a protein-restricted prepubertal rat model. Eleven melatonin analogues or metabolites were tested with the aim of evaluating the model as a test of the hypothesis that melatonin acts as a prohormone and that the ring schism metabolites (kynurenamines) mediate many of the effects attributable to melatonin. Although the hypothesis could not be confirmed, modification of the melatonin structure by lengthening the acylamide side chain or by replacing the 5 methoxy function with fluorine resulted in loss of biological potency. Modification of the melatonin structure to block the two known points of metabolism resulted in no significant alteration in biological activity. Thus 6-chloromelatonin (blocking 6-hydroxylation) and 2,3-dihydromelatonin (blocking oxidative cleavage of the C_2–C_3 bond) and 6-chloro-2,3-dihydromelatonin remained biologically active. The metabolic products of brain indoleamine-2,3-dioxygenase, *N*-acetyl-N^2-formyl-5-methoxy kynurenamine (aFoMK) and *N*-acetyl-5-methoxy kynurenamine (aMK), paradoxically were also biologically active.

Introduction

Melatonin has an important role in the timing of seasonal breeding activity in many species. In animals that mate during the increasing daylength of spring [e.g. hamsters and other, native, rodents including rats (Irby *et al.* 1984)] the pineal gland is thought to be of importance for terminating breeding activity prior to winter. In other species such as sheep, goats, deer, etc., which breed generally in autumn (shortening daylength), apparently the same pattern of secretion of melatonin initiates reproductive activity (Kennaway 1984). While there is now compelling evidence to show that the pineal hormone initiates or terminates reproductive activity by interactions with steroids at the hypothalamic/pituitary level (Karsch *et al.* 1984) the molecular basis of the interaction is unknown.

As part of a study on the mechanism of action of melatonin we have investigated some of the structural requirements for melatonin-like activity. Analogues that were predicted to be more resistant to metabolism or had functional-group changes likely to alter biological potency were prepared. Other compounds examined were intended to test the hypothesis that melatonin is a prohormone and that the brain metabolites of melatonin, *N*-acetyl-N^2-formyl-5-methoxy kynurenamine (aFoMK) and *N*-acetyl-5-methoxy kynurenamine (aMK) (Hirata *et al.* 1974), may mediate many of the effects ascribed to melatonin itself. In a previous communication we described the use of the prolactin-inhibiting activity of melatonin in sheep as our bioassay (Kennaway *et al.* 1986). In the work discussed in this report we

0004-9417/88/030393$03.00

have exploited the potent puberty-delaying effect of melatonin in male rats (Lang *et al.* 1983). The sensitivity of this assay has been enhanced by using protein-deprived prepubertal rats (Blask *et al.* 1981), thus establishing a simple, economical and reliable method for investigating some aspects of melatonin-like activity.

Methods

Male rats of the Porton strain were obtained from the University of Adelaide Central Animal House at 21 days of age in groups of 30. On arrival the rats were weighed and placed five to a cage in a light-controlled box (14 h light : 10 h dark; lights off 1200 h). The animals were fed *ad lib.* a low-protein diet (500 g powdered Kelloggs Rice Bubbles, 10 g glucose, 5 g dicalcium phosphate and 10 ml cod liver oil; Irby *et al.* 1984). This was supplemented with approximately 8 g commercial rat chow per box per day. Water was available *ad lib.* In previous experiments (Kennaway *et al.* 1988) it was found that the body weights of Porton rats maintained under these conditions were 70% of peers fed rat chow *ad lib.*

In all of the experiments, groups of five rats were injected subcutaneously with vehicle [50–100 μl ethanolic saline (10%)] or test compound in vehicle 2–3 hours before lights out between Monday and Friday. (A preliminary experiment had indicated that there was no difference in the gonadal response to melatonin when animals were injected each day of the week or simply on the 5 working days.) After 21 days (42 days old) rats were killed and seminal vesicles, ventral prostate and right testis were dissected and weighed. In a preliminary experiment to establish the assay, the effect of different doses of melatonin (1 μg, 5 μg, 10 μg, 50 μg and 100 μg per day) and the duration of treatment on the gonadal response was examined.

In Experiment 1 a comparison was made between effects of saline and comparable dosages (50 μg/day) of melatonin (aMT) and the following compounds: 6-chloromelatonin (aMT.6Cl) 2,3-dihydromelatonin (DH.aMT), 6-chloro-2,3-dihydromelatonin (DH.aMT.6Cl) and 1-methyl melatonin (aMT.1Me).

Experiment 2 examined *N*-acetyl-5-fluorotryptamine (aFT), *N*-propionyl-5-methoxytryptamine (pMT), *N*-butyryl-5-methoxytryptamine (bMT) and *N*-propionyl-5-fluorotryptamine (pFT).

Experiment 3 examined *N*-acetyl-N^2-formyl-5-methoxy kynurenamine (aFoMK; 1 mg) and *N*-acetyl-5-methoxykynurenamine (aMK; 1 mg).

Experiment 4 examined *N*-acetyl-5-bromotryptamine (aBT), aFT (300 μg), aFoMK (100 μg) and aMK (100 μg).

Determination of a significant delay in puberty (low sex and accessory gland weight) was made in each experiment by a one-way analysis of variance. Differences between the treatment groups and vehicle-treated groups were determined by the Student–Neuman–Keuls method.

Sources and Syntheses of Analogues

N-*acetyl*-N^2-*formyl-5-methoxykynurenamine*

To a stirred solution of melatonin (0·04 mol, 9·3 g) in dichloromethane (500 ml) at 4°C was added m-chloroperbenzoic acid (0·174 mol, 30·0 g). The mixture was stirred at 4°C for 16 h and washed with saturated aqueous sodium bicarbonate (3 × 500 ml). The solution was dried (Na_2SO_4) and evaporated under reduced pressure to afford a red oil (6·0 g), which was absorbed on neutral alumina grade I (150 g) with ethyl acetate. The alumina column was eluted with ethyl acetate, ethyl acetate–chloroform (1 : 1) and chloroform, and the eluant discarded. Elution with chloroform–methanol (19 : 1) afforded *N*-acetyl-N^2-formyl-5-methoxykynurenamine (2·6 g, 25%) as a yellow solid after evaporation of the solvent and washing with diethyl ether. The infra-red spectrum and the R_f value on thin layer chromatography were identical with those of a sample of the compound prepared by the method of Hirata *et al.* (1974). [Infra-red spectrum (Nujol) cm^{-1}, 1660, 1680 (C=O).]

N-*acetyl-5-methoxykynurenamine*

This compound was prepared by the method of Hirata *et al.* (1974) in 59% yield after recrystallization from isopropanol. [Infra-red spectrum (Nujol) cm^{-1}, 1640, 1665 (C=O). Mass spectrum m/z 236 (M+, 40%), 177 $(M–NH_2COCH_3)^+$, 63%), 176 (100%).]

N-acetyl-5-bromotryptamine

Oxalyl chloride (0·023 mol, 2·9 g) was slowly added to a well stirred solution of 5-bromo indole (0·02 mol, 3·9 g) in dry diethyl ether (100 ml) kept at 4°C. Following the development of an orange solution, the mixture was stirred for an additional 30 min before being filtered and washed with cold diethyl ether (80 ml). The acid chloride (yellow powder), which is stable at 0°C for several months, was suspended in toluene (100 ml) and dry ammonia gas was bubbled through the stirred mixture for 1 h. The yellow solid was filtered, washed with distilled water and dried under vacuum for 24 h to give the amide (4·7 g, 88% yield). Reduction of the amide was carried out in flame-dried glassware and in a dry nitrogen gas atmosphere in a well ventilated fumehood. The amide (0·05 mol, 1·34 g) was partially dissolved in dimethoxy ethane (50 ml) and magnetically stirred while being heated to reflux temperature for 1 h in a 100 ml three-necked flask fitted with a double-surface reflux condenser and nitrogen bubbler. Borane dimethyl sulfide (0·06 mol, 6·8 ml) was added via a syringe dropwise over 10 min into the reaction mixture. The solution was heated for 2 h and then cooled to room temperature. Methanol (20 ml) was carefully added, followed by a freshly prepared saturated dry hydrogen chloride–diethyl ether solution (5 ml). The solution was then stirred overnight at 0°C. Filtration of the solution gave 5-bromotryptamine hydrochloride [1·1 g, 79·7% yield; m.p. 288–289°C; Ho *et al.* (1969), m.p. 286–287°C]. The white amine hydrochloride (0·002 mol, 0·552 g) was partially dissolved in dimethoxy ethane–ethyl acetate (30 ml–30 ml), acetic anhydride (1 ml) and pyridine (3 drops). The solution was stirred at room temperature for 7 days under a nitrogen atmosphere. The mixture was concentrated to dryness, extracted with hot ethyl acetate (3 × 40 ml) and the extract subsequently washed with sodium hydrogen carbonate solution (2 × 30 ml), saturated sodium chloride (2 × 30 ml) and dried with anhydrous magnesium sulfate. The crude brown product was recrystallized from ethyl acetate and then chromatographed on a chromatatron using ethyl acetate–methanol (3 : 1), giving pure *N*-acetyl-5-bromotryptamine (0·31 g, 56% yield; m.p. 153·4–153·8°C). [Infra-red spectrum (KBr) cm^{-1}, 3300–3060 (N–H), 2890 (C–H), 1640 (C=O, amide), 1560, 1540, 1470, 1440, 1360, 1320, 1220, 1205, 1110, 1095, 1030, 880, 860, 790. Mass spectrum $C_{12}H_{13}N_2OBr$: m/z 282 (M+, ^{81}Br, 32%, 280 (M+, ^{79}Br, 32%), 210 [$(M(^{81}Br)\text{-}CH_2NHCOCH_3)^+$, 53%], 208 [$(M(^{79}Br)\text{-}CH_2NHCOCH_3)^+$, 59%].

Other compounds

N-acetyl 5-fluorotryptamine (aFT), *N*-propionyl 5-fluorotryptamine (pFT), *N*-propionyl 5-methoxytryptamine (pMT), and *N*-butyryl 5-methoxytryptamine (bMT) were prepared by reaction of the respective acid chloride with the corresponding 5-substituted tryptamine as follows. The tryptamine was dissolved in either DMF (5-fluorotryptamine) or ethyl acetate with a few drops of DMF (5-methoxytryptamine), with a little warming. The solution was magnetically stirred, cooled in an ice bath, and 1·1 equivalents of the acid chloride slowly added. After 15 min at 0°C, 20 μl 2M HCl was added and the solution concentrated to dryness on a rotary evaporator. The residue was extracted into acetone and worked up to yield the hydrochloride salts, which were dried under vacuum. [Infra-red spectra (KBr), and m.p.; aFT cm^{-1}, 3280 (N–H), 2600 (HCl salt), 1630 (C=O, amide), m.p. 279–281°C; pFT cm^{-1}, 3280 (N–H), 2590 (HCl salt), 1630 (C=O, amide), m.p. 277·5–279·5°C; pMT cm^{-1}, 3320 (N–H), 2590 (HCl salt), 1620 (C=O, amide), m.p. 248–250°C; bMT cm^{-1}, 3300 (N–H), 2580 (HCl salt), 1630 (C=O, amide) m.p. 233–235°C.]

6-Chloro melatonin was synthesized according to Hugel (1983), 2,3-dihydromelatonin and 6-chloro-2,3-dihydromelatonin were prepared according to Kennaway *et al.* (1986). 1-Methyl melatonin was a gift from Dr Clements, Elli Lilly and Company, and melatonin was purchased from Sigma Chemical Company.

Results

When 21-day-old male rats were maintained on a protein-restricted diet and injected with various doses of melatonin 5 days per week for 3 weeks, seminal vesicle, ventral prostate and testis weights were reduced in an apparent dose-related manner (Fig. 1). A dose of 50 μg/day for all further treatments was chosen on the basis that it was the lowest dose to give maximal reduction in the weight of all three organs. An investigation of the time course of the response confirmed that a 3-week treatment with 50 μg melatonin produced a significant response (Fig. 2).

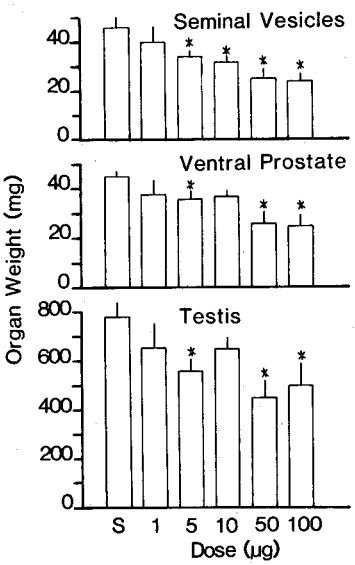


Fig. 1. Weights of seminal vesicles, ventral prostate and right testis of rats injected 5 days per week with saline (S) and various doses of melatonin between 21 and 42 days of age. Values shown are the mean s.e.m. for five animals per group. A significant decrease in organ weight is indicated by *. $P < 0{\cdot}05$ following analysis of covariance with body weight as the covariate.

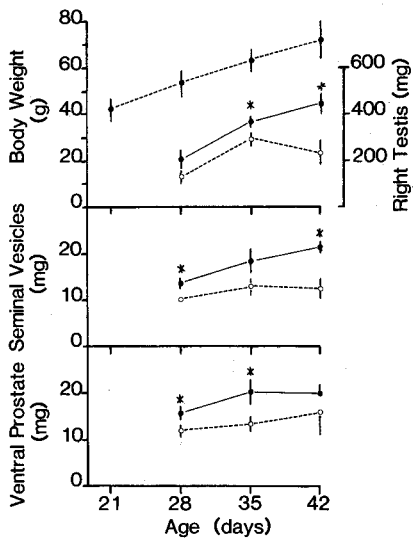


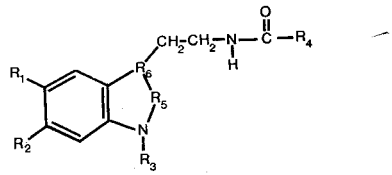
Fig. 2. Body weight, right testis, seminal vesicle and ventral prostate weights of rats injected 5 days per week with 50 μg melatonin and killed after 1, 2 or 3 weeks treatment. Values are the mean ± s.e.m. for five animals per group. Solid symbols joined by solid lines represent the saline-injected control and the open symbols joined by broken lines represent melatonin-injected group. Solid symbols joined by broken lines represents body-weight increase of both saline-treated and melatonin-treated animals, there being no significant difference between them at any age. A significant difference between control and treatment groups ($P < 0{\cdot}05$) indicated by *.

Table 1. Weights of accessory organs and testes (mg/100 g body weight mean ± s.d.) in male rats treated with melatonin (50 μg/day) between 21 and 42 days of age

	Seminal vesicles		Ventral prostate		Right testis	
	Vehicle	Melatonin	Vehicle	Melatonin	Vehicle	Melatonin
Experiment						
1	46 ± 4	25 ± 4	45 ± 2	26 ± 4	778 ± 59	451 ± 68
2	29 ± 3	17 ± 5	27 ± 7	21 ± 13	601 ± 73	323 ± 169
3	36 ± 5	18 ± 2	43 ± 3	22 ± 2	725 ± 63	376 ± 72
4	37 ± 3	24 ± 1	42 ± 4	23 ± 2	790 ± 55	490 ± 40
5	35 ± 2	22 ± 2	50 ± 6	39 ± 5	580 ± 30	350 ± 50
6	38 ± 2	21 ± 2	44 ± 5	21 ± 4	744 ± 45	360 ± 77
Mean	37 ± 5	21 ± 2	42 ± 8	25 ± 7	703 ± 90	391 ± 64
CV (%)	15	15	19	27	13	16
% Inhibition		57 ± 6		61 ± 13		56 ± 5
CV		10		22		9

Table 1 shows the weights of accessory organs and testes (mg/100 g body weight) from all six experiments in which 50 μg melatonin was injected over a 3-week period. The reduced organ weight following this treatment is very reproducible, with inter-experiment coefficients of variation less than 16% in seminal vesicle and testis weight. While the ventral prostates were always significantly lower in melatonin-treated than in vehicle-treated rats, the between-experiment variation in weight was greater. If data are presented as the percentage reduction in organ weight the between-experiment variation as shown by the coefficient of variation is greatly reduced, especially in the seminal vesicle and testis weights. Thus for ease of comparison of the activity of analogues the percentage reduction in organ weights of treated rats compared with those of control groups has been used.

Table 2. Effects of analogues of melatonin on weights of accessory organs and testes in male rats between 21 and 42 days of age



	R1	R2	R3	R4	R5	R6	Dose (μg)	Seminal vesicles (% of control)	Ventral prostate (% of control)	Testis (% of control)	Significance (*P <0·05)
Saline	—	—	—	—	—	—	—	100	100	100	
aMT	CH_3O	H	H	CH_3	-CH=	-C-	50	57	61	56	*
aFT	F	H	H	CH_3	-CH=	-C-	50	87	110	94	
							300	69	76	83	*
aBT	Br	H	H	CH_3	-CH=	-C-	50	61	76	74	*
pMT	CH_3O	H	H	CH_2CH_3	-CH=	-C-	50	82	108	90	
bMT	CH_3O	H	H	$(CH_2)_2CH_3$	-CH=	-C-	50	89	110	106	
pFT	F	H	H	CH_2CH_3	-CH=	-C-	50	105	109	95	
aMT.6Cl	CH_3O	Cl	H	CH_3	-CH=	-C-	50	69	70	52	*
aMT.1Me	CH_3O	H	CH_3	CH_3	-CH=	-C-	50	63	60	67	*
DH.aMT	CH_3O	H	H	CH_3	$-CH_2$	-C-	50	60	65	59	*
DH.aMT.6Cl	CH_3O	Cl	H	CH_3	$-CH_2$	-C-	50	46	40	50	*
aFoMK	CH_3O	H	CHO	CH_3	—	>C=O	100	67	73	72	*
							1000	53	62	68	*
aMK	CH_3O	H	H	CH_3	—	>C=O	100	78	89	93	
							1000	63	71	57	*

Table 2 summarizes the results of the four experiments in which analogues were tested. Substitution of the 5-methoxy group with fluorine or elongation of the ethylamide side chain resulted in lower biological potency. In contrast, the 5-bromo derivative was active at a dose of 50 μg/rat. Methylation of the ring nitrogen apparently failed to alter biological activity. Chlorine substitution at position 6 of the indole nucleus similarly resulted in the retention of biological activity even when accompanied by 2,3 saturation. The open-ring metabolites of melatonin (aFoMK and aMK) retained biological activity although aMK caused significant gonadal inhibition only at the high dose (1 mg).

Discussion

Daily injection of melatonin into prepubertal rats results in a time- and dose-dependent retardation of growth of the testes and accessory organs. While it is likely that this effect of melatonin is common among rodents there are strain differences; Sprague-Dawley-derived rats require some degree of food deprivation to become sensitive to melatonin (Blask *et al.* 1981) whereas Wistar rats apparently do not require this additional treatment (Lang *et al.* 1983; Kennaway *et al.* 1988). Under the conditions we employed, albino Porton rats maintained on a low-protein diet between 21 and 42 days of age had a remarkably consistent response to melatonin injection, with inter-experiment coefficients of variation being less than 16%. On this basis we considered the treatment procedure was likely to be of value for determining the structural requirements for melatonin-like activity.

The melatonin molecule was altered in four ways: (1) halogenation in the 5 position, (2) lengthening of the amide side chain, (3) halogenation in the 6 position and/or saturation of the 2,3 double bond, and (4) oxidative cleavage of the 2,3 bond.

Substitution of the 5-methoxy group with a fluorine resulted in a total loss of biological activity at 50 μg/day and partial activity at 300 μg/day. A bromine in position 5, however, resulted in almost total retention of activity when injected at a dose of 50 μg/rat. The contrasting effects of 5-fluorination and 5-bromination are not readily explained at this time. Comparison of the electronegativity of fluorine and bromine, F = 4·0, Br = 2·8 (Pauling 1960), indicates that fluorine would be more effective in the withdrawal of electrons from the aromatic ring, with resultant differences in chemical reactivity. However, the magnitude of the differences cannot be expected alone to account for the observed contrasting chemical/biological reactivities. It may be significant that the electronegative character of fluorine results in a shorter bond length, C_{sp2}–F, 1·35 Å (Lide 1962) than is the case for bromine (C_{sp2}–Br, 1·85 Å) and this coupled with the smaller size of the former (covalent radius, F = 0·71 Å, Br = 1·14 Å) could provide an explanation for their different reactivities through inductive electron withdrawal and resonance interactions with the conjugated orbitals of benzene. The possibility of *in vivo* chemoselective transformation of the 5-bromo compound cannot be ruled out.

In a previous study of the ovulation-blocking activity of melatonin analogues, acetyl, ethyl and phenyl substitutions in the 5 position resulted in total loss of activity (Flaugh *et al.* 1979). Similarly, the pencil fish pigmentation response to 5-ethoxy and 5-propyloxy derivatives was poor (Frohn *et al.* 1980). Thus, the methoxy group occupying the 5 position has special biological significance as a result of either its effect on the electron distribution of the phenyl ring structure or its physical size.

When the amide portion of the molecule was altered to produce compounds such as *N*-propionyl-5-methoxy tryptamine and the *N*-butyryl analogue (bMT), activity was lost at 50 μg/rat. This result contrasts with the very potent activity of bMT in the pencil fish assay (Frohn *et al.* 1980).

The third group of analogues represented attempts to prevent peripheral metabolism of melatonin to 6-hydroxymelatonin (Kopin *et al.* 1961) and oxidative ring cleavage in the brain to kynurenamines (Hirata *et al.* 1974). Three compounds, aMT.6 Cl, DH.aMT and DH.aMT.6 Cl, were synthesized in this group. In our assay system all three analogues were very potent blockers of puberty. We have previously shown these compounds shared with melatonin potent prolactin-lowering activity in sheep (Kennaway *et al.* 1986). Other groups have also shown that 6-chloro- and 6-fluoro- melatonin derivatives possess full biological potency in various assay systems (Flaugh *et al.* 1979; Martin *et al.* 1980; Vaughn *et al.* 1984). In the only other reported assay of 2,3-dihydromelatonin, it was found to be 10 times less potent than melatonin using the pencil fish assay (Frohn *et al.* 1980).

The fourth group of melatonin-like compounds tested were the brain metabolites of melatonin, *N*-acetyl-N^2-formyl-5-methoxykynurenamine (aFoMK) and *N*-acetyl-5-methoxykynurenamine (aMK). When injected subcutaneously at a dose of 100 μg/rat, aFoMK

caused testis and accessory sex organ weights to be depressed whereas aMK did not produce significant effects. When injected at a 10-fold higher dose both aFoMK and aMK significantly retarded reproductive maturation. This activity is in contrast to the lack of any prolactin-inhibiting activity of aFoMK following intravenous injection of 100 μg into sheep (Kennaway *et al.* 1986) and the absence of melanophore activity in cultured melanocytes of both aFoMK and aMK (Messenger and Warner 1977).

The results of this study have shown how much the melatonin 'nucleus' can be altered while still retaining various activities. ^{13}C nuclear magnetic resonance spectroscopic studies of serotonin, melatonin and analogues (Hugel, unpublished results) have shown that all these compounds have similar electron density distribution. It appears that biological activity is related to the shape and size of the molecules rather than to chemical reactivity.

In view of the hypothesis that melatonin acts as a prohormone the results obtained with dihydromelatonin and the kynurenamines do, however, pose an apparent contradiction. One possible explanation is that melatonin may have two mechanisms of action, one on the pituitary and gonadal axis that requires an intact indole structure and another central action within the hypothalamus via the 2,3 dioxygenase metabolic products. The notion that melatonin might act peripherally has had some support over recent years. The report of Peat and Kinson (1971) is of particular interest since they found a significant supression of testosterone production by melatonin *in vitro*. Such an effect *in vivo* might also partly explain melatonin-induced delayed puberty. Similarly, the melatonin inhibition of LHRH-stimulated LH secretion by neonatal rat pituitaries *in vitro* (Martin and Klein 1976) and direct inhibition of FSH and LH secretion by adult hamster pituitaries (Wun *et al.* 1986) are evidence of a peripheral effect and may contribute to the retarded growth of testicular tissue. With respect to the central actions of melatonin, numerous interactions with neurotransmitter systems have been proposed (Cardinali *et al.* 1985). Some of these effects of melatonin may be due to aFoMK and aMK. The latter metabolite has, for instance, been shown to inhibit prostaglandin synthesis (Kelly *et al.* 1984). Melatonin and aMK also show *in vitro* activity on the benzodiazepine–GABA–chloride ionophore complex (Marangos *et al.* 1981). One endocrine effect of aMK at this level may be the potentiation of the effects of GABA in neuroendocrine target areas like the pre-optic area of the brain, which is known to be a steroid-sensitive site, or the suprachiasmatic nucleus where putative melatonin-binding sites have been identified (Vanecek *et al.* 1987). In the present study the higher biological potency of aFoMK compared with aMK may simply reflect its presumed greater accessibility to the brain following subcutaneous injection due to greater lipid solubility.

In summary, a series of compounds representing various modifications of the melatonin molecule have been tested for biological activity in protein-deprived male prepubertal rats. Modifications to the molecule in the 5 position of the aromatic indole ring or on the aliphatic ethyl amine side chain nitrogen indicate that biological activity of melatonin is sensitive to chemical changes. To date our substituent effects have not shown clear and consistent structure–activity relationships. For example, the 5-bromo substituent effects are anomalous and cannot readily be rationalized. Further compounds are under investigation to clarify structure–activity relationships.

Acknowledgment

This work was supported by a grant from the National Health and Medical Research Council of Australia.

References

Blask, D. E., Nodelman, J. L., Leadem, C. A., and Richardson, B. A. (1981). Influence of exogenously administered melatonin on the reproductive system and prolactin levels in underfed male rats. *Biol. Reprod.* **22**, 507–12.

Cardinali, D. P., Vacas, M. I., and Lowenstein, P. R. (1985). Melatonin action on brain: presumptive receptors and second messengers. In 'The Pineal Gland: Current State of Pineal Research'. (Eds B. Mess, C. Ruszas, L. Tima and P. Pevet.) pp. 273–90. (Publishing House of the Hungarian Academy of Sciences: Budapest.)

Flaugh, M. E., Crowell, T. A., Clemens, T. A., and Sawyer, B. D. (1979). Synthesis and evaluation of the antiovulatory activity of a variety of melatonin analogues. *J. Med. Chem.* **22**, 63–9.

Frohn, M. A., Seaborn, C. J., Johnson, D. W., Phillipou, G., Seamark, R. F., and Matthews, C. D. (1980). Structure–activity relationships of melatonin analogues. *Life Sci.* **27**, 2043–6.

Hirata, F., Hayaishi, O., Tokuyama, T., and Senboh, S. (1974). *In vitro* and *in vivo* formation of two new metabolites of melatonin. *J. Biol. Chem.* **249**, 1311–13.

Ho, B. T., McIsaac, W. M., and Tansey, L. W. (1969). Hydroxyindole-*O*-methyltransferase III: influence of the phenyl moiety on the inhibitory activities of some *N*-acyltryptamines. *J. Pharm. Sci.* **58**, 563–6.

Hugel, H. M. (1983). Synthesis of *N*-[2-(6-chloro-5-methoxy-1H-indol-3-yl)] ethylacetamide (6-chloro-melatonin). Synthesis No. 11, 935–6.

Irby, D. C., Kerr, J. B., Risbridger, G. P., and de Kretser, D. M. (1984). Seasonally and experimentally induced changes in testicular function of the Australian bush rat (*Rattus fuscipes*). *J. Reprod. Fertil.* **70**, 657–66.

Karsch, F. J., Bittman, E. L., Foster, D. L., Goodman, R. L., Legan, S. J., and Robinson, J. E. (1984). Neuroendocrine basis of seasonal reproduction. *Recent Prog. Horm. Res.* **40**, 185–232.

Kelly, R. W., Amato, F., and Seamark, R. F. (1984). *N*-acetyl-5-methoxykynurenamine, a brain metabolite of melatonin, is a potent inhibitor of prostaglandin biosynthesis. *Biochem. Biophys. Res. Commun.* **121**, 372–9.

Kennaway, D. J. (1984). Pineal function in ungulates. *Pineal Res. Rev.* **2**, 113–40.

Kennaway, D. J., Royles, P., Dunstan, E. A., and Hugel, H. M. (1986). Prolactin response in Border-Leicester × Merino ewes to administration of melatonin, melatonin analogues, a melatonin metabolite and 6-methoxy benzoxazolinone. *Aust. J. Biol. Sci.* **39**, 427–33.

Kennaway, D. J., Royles, P., Webb, H., and Carbone, F. (1988). Effects of protein restriction, melatonin administration, and short daylength on brain benzodiazepine receptors in prepubertal male rats. *J. Pineal Res.* (in press).

Kopin, I. J., Pace, C. M. B., Axelrod, J., and Weissbach, H. (1961). The fate of melatonin in animals. *J. Biol. Chem.* **236**, 3072–5.

Lang, U., Aubert, M. L., Conne, B. S., Bradtke, J. C., and Sizonenko, P. C. (1983). Influence of exogenous melatonin on melatonin secretion and the neuroendocrine reproductive axis of intact male rats during sexual maturation. *Endocrinology* **112**, 1578–84.

Lide, D. R. (1962). A survey of carbon–carbon bond lengths. *Tetrahedron* **17**, 125–34.

Marangos, P. J., Patel, J., Hirata, F., Sondhein, D., Paul, S. M., Skolnick, P., and Goodwin, F. K. (1981). Inhibition of diazepam binding by tryptophan derivatives including melatonin and its brain metabolite *N*-acetyl-5-methoxykynurenamine. *Life Sci.* **29**, 259–67.

Martin, J. E., and Klein, D. C. (1976). Melatonin inhibition of the neonatal pituitary response to luteinising hormone, releasing hormone. *Science* **191**, 301–2.

Martin, J. E., Kirk, K. L., and Klein, D. C. (1980). Effects of 6-hydroxy-, 6-fluoro-, and 4,6-difluoro-melatonin on the *in vitro* pituitary response to luteinizing hormone releasing hormone. *Endocrinology* **106**, 398–401.

Messenger, E. A., and Warner, A. E. (1977). The action of melatonin on single amphibian pigment cells in tissue culture. *Br. J. Pharmacol.* **61**, 607–14.

Pauling, L. (1960). 'The Nature of the Chemical Bond.' 3rd Edn. (Cornell University Press: Ithaca, N.Y.)

Peat, F., and Kinson, G. A. (1971). Testicular steroidogenesis *in vitro* in the rat in response to blinding, pinealectomy and the addition of melatonin. *Steroids* **17**, 251–63.

Vanecek, J., Pavlik, A., and Illnerova, H. (1987). Hypothalamic melatonin receptor sites revealed by autoradiography. *Brain Res.* **435**, 359–62.

Vaughan, M. K., Richardson, B. A., Petterborg, L. V., Vaughan, G. M., and Reiter, R. J. (1984). Injections and/or implants of 6-chloromelatonin and melatonin: effects of plasma thyroid hormones in male and female Syrian hamsters. *Biomed. Res.* **5**, 413–18.

Wun, W-S. A., Berkowitz, A. S., and Preslock, J. P. (1986). Melatonin's differential inhibition of FSH and LH release from hamster pituitary glands. *Mol. Cell. Endocr.* **46**, 215–25.

Manuscript received 19 October 1987, revised 16 May 1988, accepted 5 July 1988