Effect of Psychotropic Drugs on the Contents of Melatonin, Serotonin and N-acetylserotonin in Rat Pineal Gland

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Accepted October 21, 1988

Abstract—In the dark phase, the effects of the psychotropic drugs on the contents of melatonin, serotonin (5-HT) and N-acetylserotonin (NAS) in rat pineal gland were examined. The pineal gland was removed at a certain period of time after subcutaneous injection of the drugs. 5-HT, NAS and melatonin contents in the pineal gland were determined by high performance liquid chromatography with fluorometric detection. A dose-dependent decrease was observed for melatonin content in the administration of diazepam (DZP), hydroxyzine (HYZ), chlorpromazine (CPZ) or haloperidol (HPD). When imipramine (IPM) or amitriptyline (APL) was given to rats, pineal 5-HT content was significantly decreased. On the other hand, the administration of IPM or APL caused increases in pineal NAS and melatonin. Furthermore, the administration of phenytoin (PYT) revealed no changes in the content of pineal indoleamines. These results suggest that the psychotropic drugs widely used in clinical applications could cause significant changes in pineal indoleamine content.

Mammalian pineal gland is considerably to be involved in various types of biological rhythm regulation and is generally considered to play a role as a neuroendocrine transducer. Attention has been focused on the pineal gland in neurochemical investigations, and various findings (1–4) have been reported in the fields of neurochemistry, physiochemistry, anatomy and clinical medicine. Melatonin is a major substance secreted by the mammalian pineal gland, and it is also enzymatically synthesized from pineal serotonin (5-HT) via N-acetylserotonin (NAS). Several reports have indicated that melatonin has physiological actions in some areas such as

the pituitary gland (5), hypothalamus (6, 7) and reproductive organs (8). The antigonadotrophic effect is the most widely recognized as the physiological action of melatonin. Melatonin has been proposed to exert its antigonadotrophic effect by acting on the hypothalamus, probably on an intrinsic neural serotonergic hypothalamic system. As another melatonin action in the hypothalamus, Kawashima et al. have suggested that melatonin acts as an endogenous anti-hypertensive factor in spontaneously hypertensive rats (7).

However, many problems still remain unsolved with regard to the physiological functions of the pineal gland and mode of action of melatonin. In order to clarify the relationship between neuropharmacological drugs and pineal function, the authors examined the effects of psychotropic agents on

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the contents of pineal indoleamines *in vivo*.

Materials and Methods

Animals: Male Wistar rats, weighing 250–300 g, were used in the experiments. Animals were housed at constant temperature ($24 \pm 1^\circ\text{C}$) and humidity ($55 \pm 5\%$) under a 12 hr light-dark cycle (lights on from 7:00 p.m. to 7:00 a.m.) for 2 weeks before the experiment. Food (MF, Oriental Yeast) and water were provided *ad libitum*.

Drugs: The following drugs were used: diazepam (DZP, Cercine inj.; Takeda Chemical Industries Co. Ltd.), hydroxyzine hydrochloride (HYZ, Atarax-P inj.; Pfizer-Taito Co. Ltd.), chlorpromazine hydrochloride (CPZ, Wintermin inj.; Shionogi Pharmaceutical Co. Ltd.), haloperidol (HPD, Serenace inj.; Dainippon Pharmaceutical Co. Ltd.), imipramine hydrochloride (IPM, Tofranil inj.; Ciba-Geigy), amitriptyline hydrochloride (APL, Lantron inj.; Yamanouchi Pharmaceutical Co. Ltd.), phenytoin (PYT, Aleviatin inj.; Dainippon Pharmaceutical Co. Ltd.). Crystalline diazepam was provided by Takeda Chemical Industries Co. Ltd.

Effect of lighting condition on the growth of rats: Male Wistar rats, weighing about 150 g, were housed under a 12 hr light-dark cycle (lights on from 7:00 p.m. to 7:00 a.m.), and animal body weight and intake of food and water were measured at 7:10 p.m. (after lights on) everyday for 2 weeks.

Effect of the lighting condition on pineal indoleamine content with the circadian rhythm: The rats were sacrificed by decapitation in the dark phase and the light phase under the lighting condition (the reversed light-dark cycle: the dark phase was set in daytime) after 3, 5, 7 and 10 days feeding, respectively. Pineals were rapidly removed, frozen on dry ice and stored at -80°C until assayed. A dim red light was used for the pineal dissection during the dark phase.

Effect of administration time of DZP on pineal indoleamine content: DZP (3 mg/kg) was administered subcutaneously to four groups ($n=5$) of rats at four different times (5:00 a.m., 6:00 a.m.: light phase; 8:00 a.m., 9:00 a.m.: dark phase). During the dark phase, the drug administration was performed under a dim red light. To the control animals ($n=5$)

were injected 0.9% saline solution with an equal volume of DZP solution at 5:00 a.m. The DZP-injected and control rats were sacrificed by decapitation at 11:00 a.m. (4 hours after the onset of the dark phase). The pineals were rapidly dissected under the dim red light, frozen on dry ice and stored at -80°C prior to HPLC analysis.

Effect of the dosage of DZP on pineal indoleamine content: DZP (0.5 to 7 mg/kg) was injected subcutaneously at 6:00 a.m. Five rats were used for each group of DZP administration. As a control ($n=20$), 0.9% saline solution was administered at 6:00 a.m. All the animals were sacrificed by decapitation at 5 hr after injection (11:00 a.m.) of DZP or saline. Pineals were rapidly removed, frozen on dry ice and stored at -80°C until the indoleamines are assayed.

Effects of the administration of HYZ, CPZ, HPD, IPM, APL and PYT on pineal indoleamine content: HYZ (3 to 6 mg/kg), CPZ (1.5 to 10 mg/kg), HPD (0.5 to 3 mg/kg), IPM (3 to 10 mg/kg), APL (1 to 6 mg/kg) and PYT (3 to 10 mg/kg) were each injected subcutaneously at 6:00 a.m. Each group of drug-administered animals consisted of five rats. The decapitation and pineal dissection were performed under the same conditions as the examination of DZP administration.

Extraction and measurement of pineal indoleamines: Extraction and measurement of indoleamines in rat pineal gland were carried out according to the previous method (9). Briefly, individual pineals were sonicated at $0-4^\circ\text{C}$ for 10 sec using a Sonicator model W-10 (Heat Systems Ultrasonics, Inc.) in 0.1 M perchloric acid containing 0.1% ascorbic acid and centrifuged at 1500 g at 0°C for 5 min. The supernatant, thus obtained, was directly injected into a high performance liquid chromatographic system (Hitachi Model 635). Pineal indoleamines were eluted with a reversed phase column (Zorbax ODS, 250×4.6 mm I.D.) using 0.01 M acetate buffer (pH 4.25) containing 35% methanol as the mobile phase and detected by an RF-530 fluorescence spectromonitor (Shimadzu) at 285 nm for excitation and 345 nm for emission. The contents of indoleamines found in the pineal were expressed as nanograms per gland.

Results

Effect of the feeding condition on the growth of rats: In order to examine the effects of psychotropic drugs on pineal indoleamine content during the dark phase in the light-dark cycle, rats were maintained under the lighting environment for 2 weeks, as shown in Fig. 1. When the dark phase in the light-dark cycle was set in the daytime, the effects of the drugs on pineal indoleamine during the dark phase could be easily examined during the reversed-daytime period. With the lighting environment completely reversed from the normal lighting cycle, the effects of changing the lighting environment on the growth of rats were examined for body weight, intake of food and water for 2 weeks as indices. The results are shown in Fig. 2. Average increase of rat body weight was about 8 g/day. Average daily intake was about 100 g/kg/day for food and about 110

ml/kg/day for water, respectively.

Effect of feeding condition on pineal indoleamine content with the circadian rhythm: It was evident that the circadian rhythm of pineal indoleamine would have been influenced by the change of the lighting environment, because rats were kept under the reversed light-dark cycle (Fig. 1). After feeding for 3, 5, 7 and 10 days, the rats were sacrificed by decapitation in the dark phase and the light phase, respectively, and then the pineal indoleamines were determined. As a result, under the reversed light-dark cycle normal circadian rhythm was recognized from the melatonin and 5-HT contents after 7 days feeding. Figure 3 shows the contents of pineal melatonin and 5-HT during the dark phase after 7 days feeding. Melatonin content was the highest at 4 hours after the onset of the dark phase (i.e., at 11:00 a.m.), whereas a low level of 5-HT was observed at 4 hours after the light was turned

Fig. 1. The lighting schedule. Animals were maintained in a 12 hr light-dark cycle (lights on from 7:00 p.m. to 7:00 a.m.) for 2 weeks.

Fig. 2. Body weight (●) and the intake of food (○) and water (▲) in male rats under the reversed light-dark cycle.

out and this level persisted for 4 more hours.

Effect of administration time of DZP on pineal indoleamine content: The effects of the administration time of DZP on pineal indoleamine content are seen clearly in the melatonin level. The 5:00 a.m. administration group showed a significant decrease (53%) compared to the control group ($P < 0.01$), and the 6:00 a.m. group showed a significant decrease at about 60% ($P < 0.01$). Also, about a 20% decrease in the melatonin content was observed in the 8:00 a.m. group compared with the control ($P < 0.05$), whereas the 9:00 a.m. group showed a decreasing tendency, but no significant difference was recognized

Fig. 3. Concentrations of melatonin (■) and serotonin (●) in rat pineal gland during the dark phase in the reversed light-dark cycle. Each point represents the mean of 5 rats. The vertical lines represent the S.E.

(Fig. 4). Figure 4 reveals that the most distinguished effects of administration time of DZP on the melatonin content appeared when DZP was given at 6:00 a.m. Therefore, the administration time of DZP was set at 6:00 a.m., and the extraction of the pineal gland was carried out at 5 hours after the injection (i.e., at 11:00 a.m.). As a check, the effect of the vehicle for Cercine injection was examined. Ten mg of crystalline diazepam was suspended in 4 ml of 0.9% saline containing 50% ethylene glycol, and the suspension was injected subcutaneously at 6:00 a.m.; the dose was 3 mg/kg. The contents of pineal indoleamines in the rats after injection of DZP suspension were almost the same as those of the Cercine-administered rats.

Effect of the dosage of DZP on pineal indoleamine content: The results (Fig. 5) show a significant decrease in the melatonin content for each of the varying dosages. The pineal melatonin content of the 0.5 mg/kg administration group showed a 30% decrease as compared with the control group ($P < 0.01$). The decrease of melatonin content was 43% for the 1 mg/kg administration group ($P < 0.01$), 58% for the 5 mg/kg group ($P < 0.01$) and 85% for the 7 mg/kg group ($P < 0.01$).

Effects of the administration of HYZ, CPZ, HPD, IPM, APL and PYT on pineal indoleamine content: Under the same conditions as the experiment on the effects of DZP administration, we examined the effects of the

Fig. 4. Pineal melatonin (MEL), serotonin (5-HT) and N-acetylserotonin (NAS) levels in rats given a subcutaneous injection of diazepam (3 mg/kg) at four different times. Each column shows the mean $\pm$ S.E. of 5 rats. * $P < 0.05$, ** $P < 0.01$, significant difference from the control.

Fig. 5. Effect of diazepam on the indoleamine contents in rat pineal gland. Each column shows the mean $\pm$ S.E. of 5 rats (Cont.: $n=20$). * $P<0.01$, significant difference from the control.

Fig. 6. Effect of hydroxyzine on the indoleamine contents in rat pineal gland. Each column shows the mean $\pm$ S.E. of 5 rats (Cont.: $n=20$). * $P<0.01$, significant difference from the control.

administration of some psychotropic drugs: HYZ (an anti-anxiety drug similar to DZP), CPZ and HPD (neuroleptics), IPM and APL (tricyclic antidepressants), and PYT (antiepileptics). Administration of HYZ led to a decrease of pineal melatonin content, similar to the case of DZP, and a significant decrease of 70% ($P<0.01$) was observed in the 6 mg/kg group as compared to the control (Fig. 6). When CPZ was given (Fig. 7), the melatonin content was also decreased similarly to the case of DZP, and a 57% decrease was observed in the 10 mg/kg administration group compared with the control value of melatonin ($P<0.01$). Similar

to the cases of DZP, HYZ and CPZ, only the melatonin content was decreased by HPD injection (Fig. 8). Consequently, a significant decrease of 80% was noted in the 2 mg/kg administration group compared with the control ($P<0.01$). The variations of pineal indoleamines, when IPM, APL and PYT were given, are shown in Table 1. Unlike the results mentioned above for the 4 other drugs, when IPM or APL was injected, pineal 5-HT content decreased, while NAS and melatonin contents increased. On the other hand, administration of PYT did not significantly alter the contents of pineal 5-HT, NAS and melatonin during the dark phase.

Fig. 7. Effect of chlorpromazine on the indoleamine contents in rat pineal gland. Each column shows the mean $\pm$ S.E. of 5 rats (Cont.: n=20). *P<0.01, significant difference from the control.

Fig. 8. Effect of haloperidol on the indoleamine contents in rat pineal gland. Each column shows the mean $\pm$ S.E. of 5 rats (Cont.: n=20). *P<0.01, significant difference from the control.

Table 1. Effect of psychotropic drugs on the indoleamine contents in rat pineal gland

Drugs	Doses mg/kg, s.c.	No. of animals	Content (ng/pineal gland)		
			Serotonin	N-acetylserotonin	Melatonin
Control		20	54.8 $\pm$ 9.7	5.37 $\pm$ 1.45	2.80 $\pm$ 0.31
Imipramine	3.0	5	31.5 $\pm$ 5.7***	7.29 $\pm$ 2.38*	3.54 $\pm$ 0.49***
	6.0	5	28.3 $\pm$ 6.6***	8.11 $\pm$ 3.56**	3.58 $\pm$ 0.36***
	10.0	5	17.8 $\pm$ 5.2***	8.92 $\pm$ 3.19***	4.02 $\pm$ 0.61***
Amitriptyline	1.0	5	34.7 $\pm$ 5.4***	6.56 $\pm$ 1.69	4.18 $\pm$ 0.67***
	3.0	5	22.1 $\pm$ 6.4***	8.67 $\pm$ 1.15***	4.40 $\pm$ 1.03***
	6.0	5	26.6 $\pm$ 5.5***	8.52 $\pm$ 2.62***	4.58 $\pm$ 0.56***
Phenytoin	3.0	5	50.6 $\pm$ 9.4	5.24 $\pm$ 1.32	2.92 $\pm$ 0.32
	10.0	5	54.4 $\pm$ 7.8	5.58 $\pm$ 1.89	2.51 $\pm$ 0.40

Each value shows the mean $\pm$ S.E. *P<0.05, **P<0.02, ***P<0.01: Significant difference from the control.

Discussion

Since Deguchi and Axelrod (10) have reported the sensitive determination of pineal N-acetyltransferase activity, many attempts have been made to examine the relationship between the pineal function and adrenergic (11), adrenergic blocking (12), antidepressant (13, 14) and anti-inflammatory drugs (15). In the present study, in order to clarify the relationship between the pineal function and psychotropic drug action, we examined the effects of various psychotropic agents on the content of rat pineal indoleamine during the dark phase. Melatonin is a major pineal indoleamine, and its synthesis in rat pineal gland is enhanced during the dark phase by the following pathway: 1) In the dark phase, norepinephrine (NE) is released from the terminals of nerves in the superior cervical ganglia. 2) The released NE acts on the membranes of the pinealocytes interacting with pineal beta receptors, resulting in an increase of intracellular cyclic AMP. 3) The increased cyclic AMP mediates the activation of N-acetyltransferase (NAT). 4) NAT converts 5-HT to NAS. 5) Hydroxyindole-O-methyltransferase (HIOMT) exerts its action on NAS, resulting in biosynthesis of melatonin. Accordingly, the advantage of an investigation under the dark phase is that it allows an overall assessment of the effects of drug administration on the pineal indoleamine contents. In this study, the dark phase in the light-dark cycle was set in the daytime to make full use of the advantage described above during the daytime. The levels of pineal indoleamines extensively fluctuate in response to light stimulation (16), and the melatonin content is influenced by various types of stress (17–19). Considering these influences, it is essential to examine the effect of a change in the feeding environment on the indoleamine content in rat pineal gland. At first, we studied the physical growth of rats under a reversed lighting cycle. A steady increase in rat body weight was observed under the reversed lighting cycle, and the intake of food and water was also constant (Fig. 2). By confirming the progress of normal physical development, we concluded that the rats were not subjected to

stress induced by the change in the feeding environment. Furthermore, circadian rhythms of pineal 5-HT and melatonin were recognized in the reversed lighting cycle, and the contents of both indoleamines in the pineal were not different from those previously reported values (20–22). From the above observations, we inferred that there was no effect on the content of pineal indoleamines by changing the feeding environment. Next, we examined the effects of psychotropic drugs on pineal indoleamines.

In this study, IPM or APL decreased the pineal 5-HT level, while both drugs increased NAS and melatonin contents in rat pineal gland (Table 1). These results suggest that NE released from the terminals of nerves in the superior cervical ganglia was increased by the action of these two drugs which inhibit NE reuptake, and the released NE in the terminals of nerves stimulated the synthesis of NAS and melatonin. Friedman et al. reported that acute treatment (3 days) of rats with IPM elicited increases of pineal 5-HT, NAS and melatonin (14). Comparing the results of IPM administration with the data reported by Friedman et al. (14), the variation of pineal 5-HT was observed. No difference was observed for melatonin and NAS content in both results, whereas an increase of serotonin was found by Friedman and a decrease observed in our report. This conflicting data on pineal 5-HT level may be caused by differences in the dosage and administration time of IPM.

When the antiepileptic PYT was administered, pineal indoleamine content during the dark phase was not affected. Morton et al. have reported that the administration of PYT to rats induced decreases of the NAT and HIOMT activities during the light phase (23). As might be suspected, the decrease of the contents of pineal NAS and melatonin can be attributed to the reduction of the NAT and HIOMT activities. These observations, therefore, may suggest that the effects of the drugs on the contents of pineal indoleamines differ in each phase of the light-dark cycle.

The most interesting findings in our present study are as follows: the effects of DZP, HYZ, CPZ and HPD on pineal indoleamine contents were noted only in melatonin level,

and all these drugs caused a significant decrease in melatonin during the dark phase (Figs. 4–8). From the results, the following may be presumed: 1) The administration of the above four drugs will enhance the secretion and the metabolism of melatonin. Sugden (24) and Lieberman et al. (25) reported that a significant and short time sedative effect was observed by the administration of melatonin to man or rat, whereas for animals (26) or humans (27), melatonin acts as an effective hypnotic agent. Therefore, melatonin has sedative and anticonvulsant effects similar to the actions of DZP, HYZ, CPZ and HPD. By the administration of DZP, HYZ, CPZ and HPD, pineal melatonin content decreases, probably because of melatonin secretion from the pineal glands. The action of melatonin will be accelerated with the administration of these four drugs. 2) The administration of the four drugs may reduce the pineal HIOMT activity. HIOMT activity in rat pineal gland is reduced by indomethacin administration (28). The neuroleptics, CPZ and HPD, reduce the HIOMT activity in vitro (29). From these findings, CPZ, HPD, DZP and HYZ may also cause a reduction of pineal HIOMT activity in vivo, as similarly reported for CPZ and HPD in vitro. 3) Suprachiasmatic nuclei (SCN) in the hypothalamus are a principal pacemaker controlling the circadian rhythm of the pineal indoleamines (30). Since the functions of SCN in the hypothalamus are suppressed by the administration of any of the four drugs, the content of pineal melatonin should be decreased. Zatz et al. have reported that the i.p. administration of high doses of central depressants blocked the nocturnal rise of rat pineal NAT activity by acting on the SCN (31). Chronic treatment with lithium (13) or IPM (14) suppressed the dark-induced elevation of pineal NAT activity, and led to decreases in the contents of pineal NAS and melatonin. These studies suggest that the pineal NAT activity, NAS and melatonin contents decreased by the drug-induced suppression of SCN. In order to demonstrate the drug-induced suppression of SCN, resulting in the significant decrease of pineal melatonin content at 5 hours after DZP, HYZ, CPZ and HPD administrations, we

have to ascertain the changes of NAT activity, NAS and melatonin contents up to 5 hours after the four drug administrations. In addition to the presumptions described above, the results obtained from these four drugs would have been brought about by these combined presumptions.

In this paper, the administration of DZP, HYZ, CPZ and HPD to rats caused decreases of pineal melatonin content. The decrease of pineal melatonin content would have some influence, particularly on the reproductive organ or endocrine system. The hypertrophy of reproductive organs (32) and the increase of luteinizing or follicle-stimulating hormone levels in the serum (33, 34) would be observed if the decrease of pineal melatonin content is continued. Further detailed studies in vivo and in vitro should be made to measure NAT and HIOMT activities in order to clarify the action of psychotropic drugs on rat pineal gland.

In conclusion, we have found that the administration of the psychotropic drugs widely used in clinical applications brought about significant changes in rat pineal indoleamine content during the dark phase. In particular, administration of DZP, HYZ, CPZ and HPD caused a dose-dependent decrease in the maximum melatonin levels.

Acknowledgment: The authors express appreciation to Takeda Chemical Industries Co., Ltd. for kindly supplying crystalline diazepam.

References

- 1 Erlich, S.S. and Apuzzo, M.L.J.: The pineal gland: Anatomy, physiology and clinical significance. *J. Neurosurg.* **63**, 321–341 (1985)
- 2 Wetterberg, L.: Melatonin in humans physiological and clinical studies. *J. Neural Transm. [Suppl.]* **13**, 289–310 (1978)
- 3 Reiter, R.J.: Normal patterns of melatonin levels in the pineal gland and body fluids of humans and experimental animals. *J. Neural Transm. [Suppl.]* **21**, 35–54 (1986)
- 4 Ebadi, M. and Govitrapong, P.: Neural pathways and neurotransmitters affecting melatonin synthesis. *J. Neural Transm. [Suppl.]* **21**, 125–155 (1986)
- 5 Smythe, G.A. and Lazarus, L.: Growth hormone regulation by melatonin and serotonin. *Nature* **224**, 230–231 (1973)
- 6 Chrousos, G.P., Brown, T. and Bercu, B.B.:

- Pharmacologic effects of melatonin on hypothalamic-adenohypophyseal function in the nonhuman primate. *Neuroendocrinology* **34**, 343–346 (1982)
- 7 Kawashima, K., Miwa, Y., Fujimoto, K., Oohata, H., Nishino, H. and Koike, H.: Participation of melatonin in the regulation of blood pressure in spontaneously hypertensive rats. *In* Brain and Blood Pressure Control, Edited by Nakamura, K., p. 243–248, Elsevier Amsterdam (1986)
- 8 Pévet, P. and Haldar-Misra, C.: Effect of orally administered melatonin on reproductive function of the golden hamster. *Experientia* **38**, 1493–1494 (1982)
- 9 Wakabayashi, H., Shimada, K. and Aizawa, Y.: Variation of melatonin and serotonin content in rat pineal gland with sex and oestrous phase difference determined by high-performance liquid chromatography with fluorimetric detection. *J. Chromatogr.* **381**, 21–28 (1986)
- 10 Deguchi, T. and Axelrod, J.: Sensitive assay of serotonin N-acetyltransferase activity in rat pineal. *Anal. Biochem.* **50**, 174–179 (1972)
- 11 Deguchi, T. and Axelrod, J.: Induction and superinduction of serotonin N-acetyltransferase by adrenergic drugs and denervation in rat pineal organ. *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2208–2211 (1972)
- 12 Lipton, J.S., Petterborg, L.J. and Reiter, R.J.: Influence of propranolol, phenoxybenzamine or phentolamine on the in vivo nocturnal rise of pineal melatonin levels in the Syrian hamster. *Life Sci.* **28**, 2377–2382 (1981)
- 13 Yocca, F.D., Lynch, V.P. and Friedman, E.: Effect of chronic lithium treatment on rat pineal rhythms: N-acetyltransferase, N-acetylserotonin and melatonin. *J. Pharmacol. Exp. Ther.* **226**, 733–737 (1983)
- 14 Friedman, E., Yocca, F.D. and Cooper, T.B.: Antidepressant drugs with varying pharmacological profiles alter rat pineal beta adrenergic-mediated function. *J. Pharmacol. Exp. Ther.* **228**, 545–550 (1984)
- 15 Reiter, R.J., Steinlechner, S. and Richardson, B.A.: Daytime and nighttime pineal N-acetyltransferase activity and melatonin content in male rats treated with indomethacin, a prostaglandin synthesis inhibitor. *Neuroendocrinol. Lett.* **7**, 281–287 (1985)
- 16 Deguchi, T. and Axelrod, J.: Control of circadian change of serotonin N-acetyltransferase activity in pineal by the β -adrenergic receptor. *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2547–2550 (1972)
- 17 Yocca, F.D. and Friedman, E.: Effect of immobilization stress on rat pineal β -adrenergic receptor-mediated function. *J. Neurochem.* **42**, 1427–1432 (1984)
- 18 Oxenkrug, G.F. and McIntyre, I.M.: Stress-induced synthesis of melatonin: Possible involvement of the endogenous monoamine oxidase inhibitor (Tribulin). *Life Sci.* **37**, 1743–1746 (1985)
- 19 Lynch, H.J. and Deng, M.H.: Pineal responses to stress. *J. Neural Transm. [Suppl.]* **21**, 461–473 (1986)
- 20 Wakabayashi, H., Shimada, K. and Aizawa, Y.: Determination of serotonin and melatonin in rat pineal gland by high-performance liquid chromatography with ultraviolet and fluorometric dual detection. *Chem. Pharm. Bull. (Tokyo)* **33**, 3875–3880 (1985)
- 21 Snyder, S.H., Axelrod, J. and Zweig, M.: Circadian rhythm in the serotonin content of the rat pineal gland: Regulating factors. *J. Pharmacol. Exp. Ther.* **158**, 206–213 (1967)
- 22 Young, S.N. and Anderson, G.M.: Factors influencing melatonin, 5-hydroxytryptophol, 5-hydroxyindoleacetic acid, 5-hydroxytryptamine and tryptophan in rat pineal glands. *Neuroendocrinology* **35**, 464–468 (1982)
- 23 Morton, D.J.: Effect of anticonvulsant drugs in vivo on rat pineal N-acetyltransferase and hydroxyindole-O-methyltransferase. *J. Pineal Res.* **3**, 181–186 (1986)
- 24 Sugden, D.: Psychopharmacological effects of melatonin in mouse and rat. *J. Pharmacol. Exp. Ther.* **227**, 587–591 (1983)
- 25 Lieberman, H.R., Waldhauser, F., Garfield, G., Lynch, H.J. and Wurtman, R.J.: Effects of melatonin on human mood and performance. *Brain Res.* **323**, 201–207 (1984)
- 26 Marczyński, T., Yamaguchi, P.N., Ling, G.M. and Grodzinska, L.: Sleep induced by the administration of melatonin to the hypothalamus of unrestrained cats. *Experientia* **20**, 435–437 (1964)
- 27 Cramer, H., Rudolf, J., Consbruch, U. and Kendel, K.: On the effects of melatonin on sleep and behavior in man. *Adv. Biochem. Psychopharmacol.* **11**, 187–191 (1974)
- 28 Ritta, M.N. and Cardinali, D.P.: Effect of indomethacin on monoamine metabolism and melatonin synthesis in rat pineal gland. *Hormone Res.* **12**, 305–312 (1980)
- 29 Cremer-Bartels, G., Ebels, I., Sykes, J.E.C. and De Moree, A.: Effects of retinal and pineal low molecular weight fractions and antipsychotic drugs on hydroxyindole-O-methyltransferase. *J. Neural Transm.* **58**, 107–119 (1983)
- 30 Klein, D.C. and Moore, R.G.: Pineal N-acetyl-

- transferase and hydroxyindole-O-methyltransferase: Control by retinohypothalamic tract and the suprachiasmatic nucleus. *Brain Res.* **174**, 245–262 (1979)
- 31 **Zatz, M. and Brownstein, M.J.:** Central depressants rapidly reduce nocturnal serotonin N-acetyltransferase activity in the rat pineal gland. *Brain Res.* **160**, 381–385 (1979)
- 32 **Motta, M., Fraschini, F. and Martini, L.:** Endocrine effects of pineal gland and of melatonin. *Proc. Soc. Exp. Biol. Med.* **126**, 431–435 (1967)
- 33 **Alonso, R., Prieto, L., Hernandez, C. and Mas, M.:** Antiandrogenic effects of the pineal gland and melatonin in castrated and intact prepubertal male rats. *J. Endocrinol.* **79**, 77–83 (1978)
- 34 **Jayatilak, P.G. and Sheth, A.R.:** Enhancement of serum FSH levels after pinealectomy. *Experientia* **36**, 262–263 (1980)