Brit. J. Pharmacol. (1965), **24**, 455–461.

EFFECT OF MELATONIN ON SPONTANEOUS CONTRACTIONS AND RESPONSE TO 5-HYDROXYTRYPTAMINE OF RAT ISOLATED DUODENUM

BY

M. R. QUASTEL* AND R. RAHAMIMOFF

From the Rogoff Laboratory of Physiology, the Hebrew University—Hadassah Medical School, Jerusalem, Israel

(*Received July* 28, 1964)

The isolation of melatonin from the pineal gland (Lerner, Case, Takahashi, Lee & Mori, 1958; Lerner, Case & Takahashi, 1960) has led to increased speculation regarding the hormonal role of this substance. The *o*-methylating enzyme (hydroxyindole-*o*-methyl transferase) appears to be located only in the pineal gland (Axelrod, Maclean, Alberts & Weissbach, 1961), whereas melatonin (*N*-acetyl-5-methoxytryptamine) has been detected in peripheral nerves (Lerner, Case, Mori & Wright, 1959) which may concentrate it from the bloodstream (Wurtman, Axelrod & Potter, 1964). Equally, the small intestine and other organs have been shown capable of concentrating tritium-labelled melatonin from the circulation (Kopin, Pare, Axelrod & Weissbach, 1961). Little is known of the physiological and pharmacological properties of this substance, although it has been found to be a very potent aggregator of melanophore pigment granules, causing the bleaching of frog skin (Lerner *et al.*, 1958).

Melatonin is similar in structure to 5-hydroxytryptamine from which it may be derived (Axelrod & Weissbach, 1960; Weissbach, Redfield & Axelrod, 1960). 5-Hydroxytryptamine has been shown to be present in the pineal gland (Giarman, Freedman & Picard-Ami, 1960), but it also exists in large amounts in the gut (Erspamer & Asero, 1952) where it is possibly of importance in the control of peristalsis (Bülbring, 1961). In view of the similar chemical structure, metabolic interrelationship and intimate association of these two substances in the pineal gland, it was thought desirable to examine the effects of melatonin on the contractile properties of intestinal smooth muscle.

METHODS

Female rats of the Sabra strain, bred for 30 years at the Hebrew University, were used in all experiments. Ranging in weight from 150 to 250 g, they were housed in metal cages at approximately 20° C and were fed *ad libitum* on water and food pellets (prepared by the Experimental Unit of the Animal House, Hebrew University, Jerusalem). At 30 sec after killing by stunning and cervical fracture, duodenal segments 2 to 3 cm long were removed at a distance of 2 to 4 cm from the pylorus and placed immediately in cold

* Present address: Radiation Protection Division, Department of National Health and Welfare, Ottawa, Ontario, Canada.

Krebs-Ringer-bicarbonate solution (Umbreit, Burris & Stauffer, 1957) to which 1 mg/ml. of glucose had been added. The specimens were observed to contract during the next few minutes to 1.5 to 2.2 cm in length. The segments were then attached by threads to a simple lever-kymograph arrangement and suspended vertically in the medium through which 5% carbon dioxide and 95% oxygen were bubbled at 37° C Length changes were recorded isotonically.

Spontaneous contractions began within several seconds after immersion in the warm medium and were accompanied by an increase in length of the specimens. The pieces of gut were left for about 30 min before experimental observations were started. During this time a steady state in the tone and spontaneous contraction amplitude generally became established and usually lasted for the next hour or longer.

Melatonin (Regis Chemical Co., 1219 N. Wells Street, Chicago, Illinois, U.S.A.) and 5-hydroxytryptamine creatine sulphate (L. Light & Co., Colnbrook), were dissolved in the medium at appropriate concentrations. Melatonin doses ranged from 1.25 to 300×10^{-9} moles/ml. while the 5-hydroxytryptamine concentrations were between 0.01 and 0.8×10^{-9} moles/ml. Three types of experiment were carried out. First, the melatonin was given alone in gradually increasing concentrations, between which the medium was washed out and replaced with fresh Krebs-Ringer-bicarbonate solution. After washing out, the preparation was allowed to contract spontaneously for 10 to 15 min before further melatonin was added. In the second type of experiment, melatonin and 5-hydroxytryptamine were added to the preparations simultaneously. The third type consisted of adding 5-hydroxytryptamine and after 2 min pipetting melatonin solution into the medium surrounding the specimen. The percentage inhibition by melatonin of the initial 5-hydroxytryptamine-induced contractions was measured from the smoked paper records and dose/response curves were constructed. All the following observations refer to events immediately and shortly after the administration of melatonin to the intestinal segments.

RESULTS

Effect of melatonin on spontaneous intestinal contractions

An immediate and marked inhibition was observed. The effect was dose-dependent. A very slight effect was observed at 1.25×10^{-9} moles/ml., whereas almost complete inhibition was obtained at 160×10^{-9} moles/ml. The suppression of motility is shown in the tracing of Fig. 1. An inhibition of 50% of the mean contraction amplitude was brought about by 5 to 10×10^{-9} moles/ml. of melatonin (Fig. 1). The frequency of contractions remained unchanged after the administration of melatonin, as shown in Table 1 and Fig. 2.

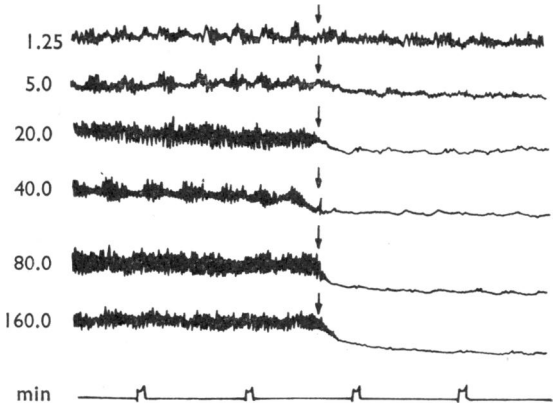

Fig. 1. Effect of various melatonin concentrations (figures on left, moles/ml. $\times10^{-9}$) on spontaneous duodenal contractions *in vitro*. Arrows indicate time of administration.

TABLE 1

EFFECT OF MELATONIN ON SPONTANEOUS CONTRACTION AMPLITUDE AND RATE OF INTESTINAL SEGMENTS *IN VITRO*

Average amplitude of spontaneous contractions was measured during 1 min immediately before and after administration of melatonin, and values are means of two experiments. Contractions were measured during 3-min periods before and after melatonin and are means within ranges of ± 1 per min. ± values are maximum ranges for both experiments.

Dose (moles/ml. $\times 10^{-9}$)	Contractions per minute		Inhibition of amplitude (%)
	Before melatonin	After melatonin	
1·25	35	34·5	22 ±13
5·0	37	37	51 ± 7
10·0	33	34	56 ± 7
20·0	35	35	81 ± 8
40·0	36·5	36	82·5 ± 5
80·0	36	37	92 ± 3
160·0	36	37	96 ± 3

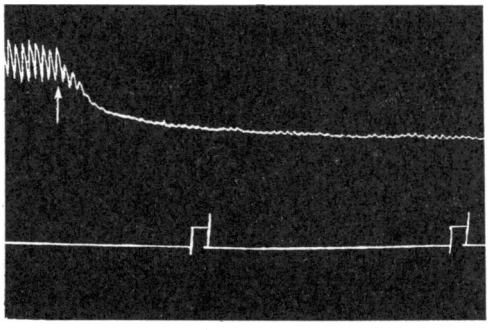

Fig. 2. Effect of melatonin (160×10^{-9} moles/ml., at arrow) on spontaneous duodenal contractions. Inhibition began 1 to 2 sec after administration. Initial strong contractions are 4% of length of intestinal segment. Time marks are 1 min apart.

Effect of melatonin on the intestinal motile response to 5-hydroxytryptamine

Because of the similar chemical structures of melatonin and 5-hydroxytryptamine, it was thought possible that the observed inhibition was brought about by competition with intestinal 5-hydroxytryptamine. The gut was therefore caused to contract with 5-hydroxytryptamine and the effect of melatonin on this response was studied. When

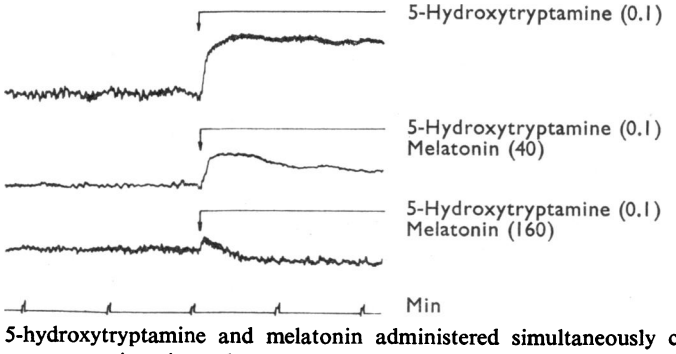

Fig. 3. Effect of 5-hydroxytryptamine and melatonin administered simultaneously compared to the effect of 5-hydroxytryptamine given alone. Upward deflection indicates longitudinal contraction of the specimen. Doses in moles/ml. $\times 10^{-9}$.

added to the medium alone, 5-hydroxytryptamine caused a marked contraction which relaxed only gradually. However, when melatonin was added along with the same amount of 5-hydroxytryptamine, the amplitude of the resultant contraction was much smaller and fell more rapidly to the levels observed before giving the drug (Fig. 3).

The inhibitory effect of melatonin, when given 2 min after the motile response of the intestinal specimen to 5-hydroxytryptamine, is shown by the kymograph tracing in Fig. 4. After the addition of 5-hydroxytryptamine, subsequent administration of melatonin caused

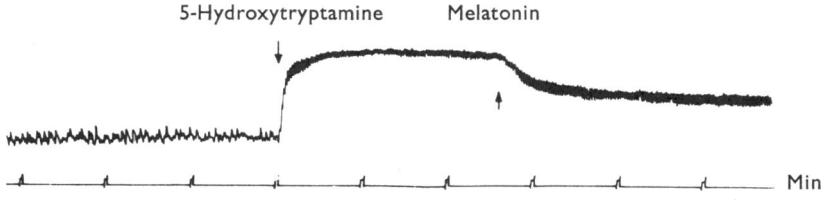

Fig. 4. Effect of melatonin (40×10^{-9} moles/ml., at second arrow) on the motile response of a duodenal specimen to 5-hydroxytryptamine (0.1×10^{-9} moles/ml., at first arrow).

a reduction in tone (represented isotonically by an increase in length), but did not necessarily result in a suppression of contraction amplitude, unless larger amounts of the latter drug were used (Fig. 4).

The dose/response curves in Fig. 5 illustrate the suppression induced by melatonin when added 2 min after the contractile response to various concentrations of 5-hydroxytryptamine. It is apparent that melatonin was much more effective at low 5-hydroxytryptamine

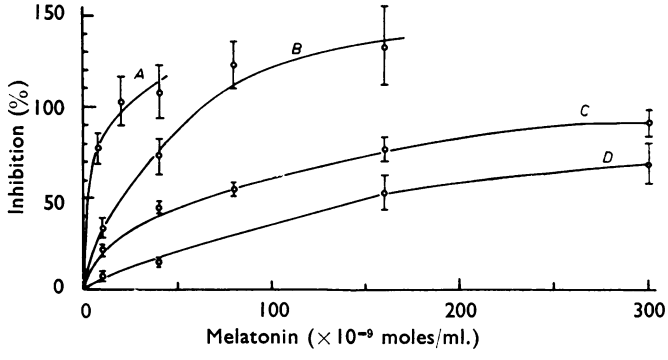

Fig. 5. Percentage inhibition by melatonin of 5-hydroxytryptamine-induced contractions. 5-Hydroxytryptamine concentrations ($\times 10^{-9}$ moles/ml.): *A*, 0.01; *B*, 0.1; *C*, 0.4; and *D*, 0.8. Vertical lines give standard deviations.

doses and less potent at higher values. An inhibition of more than 100% indicates that, after giving melatonin, the final length of the specimen was greater than that before the administration of 5-hydroxytryptamine.

The concentrations of melatonin required to cause 50% inhibition of the shortening due to various doses of 5-hydroxytryptamine are plotted in Fig. 6. From the slope of the linear relationship obtained, it was determined that, under the prevailing experimental conditions, a molecular ratio of 170 : 1 for melatonin : 5-hydroxytryptamine was necessary to bring about this degree of inhibition.

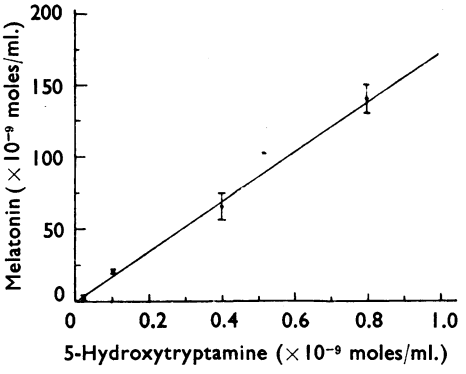

Fig. 6. Melatonin concentrations required for 50% inhibition of the shortening caused by various doses of 5-hydroxytryptamine. Vertical lines give standard deviations.

These observations with 5-hydroxytryptamine are in contrast to preliminary findings with acetylcholine (Quastel & Rahamimoff, unpublished). Melatonin also partially inhibited acetylcholine-induced longitudinal contractions of rat duodenum. However, for the same contraction amplitude as obtained with 5-hydroxytryptamine, an inhibition of 50%, if attained, required melatonin concentrations of a higher order of magnitude.

DISCUSSION

Our results demonstrate that melatonin induces *in vitro* a marked inhibition of the spontaneous contractions and motile response of the rat duodenum to 5-hydroxytryptamine. The linearity of the relationship between the dose of 5-hydroxytryptamine and the concentration of melatonin required for 50% inhibition is suggestive of competition. However, the available results do not allow one to conclude that the two substances are necessarily acting on the same receptor sites. Furthermore, because of the relatively large molecular ratio (170 : 1) for 50% suppression, it would seem unlikely that the inhibition observed *in vitro* represents a physiological mechanism for the inhibition of intestinal contractions.

The reported observations are not in agreement with those of Kappers (1962) who was unable to elicit, with melatonin, a suppression of the contractile response of the ileum or oestrous uterus to 5-hydroxytryptamine. In the preliminary report of the latter work, the doses used were unspecified and it is therefore difficult to make comparisons with the present results.

It is of interest that, while the contractile frequency remained unchanged after melatonin, which would indicate that the rhythmic pacemaker activity of the intestinal musculature was uninfluenced by the drug, the amplitude of the duodenal contractions and/or tone were strongly affected. This might have been due either to impaired contractile ability of the smooth muscle fibres or to inhibition of transmission through the intestinal musculature. It may be significant in this respect that Rahamimoff (unpublished) has found that melatonin reduced the sensitivity of peripheral nerve.

With these observations the number of apparently unrelated properties of melatonin is increased. In addition to its potent bleaching action on frog skin melanophores (Lerner *et al.*, 1958), melatonin has been found to have an inhibitory effect on the response of the

thyroid to methylthiouracil (Baschieri, De Luca, Cramarossa, De Martino, Olivario & Negri, 1963), on the development of rat seminal vesicles (Kappers, 1962) and, when given in minute doses for long periods, on ovarian growth and oestrus (Wurtman, Axelrod & Chu, 1963). The sensitivity to light of the size (Wurtman, Roth, Altschule & Wurtman, 1961; Fiske, Pound & Putman, 1962), histological structure (Roth, Wurtman & Altschule, 1962) and chemistry (Quay, 1961, 1963; Quay & Halevy, 1962) of the pineal gland has been related to melatonin synthesis by the finding (Wurtman, Axelrod & Fischer, 1964; Wurtman, Axelrod & Potter, 1964) that pineal hydroxyindole-*o*-methyl transferase activity is also markedly influenced by exposure to light.

The significance of the present observations may lie in the fact that melatonin is a naturally occurring substance, whereas most of the extensive number of agents with properties antagonistic to 5-hydroxytryptamine (Gyermek, 1961) are of an extrinsic nature. Further work is required on the effects of melatonin on the peripheral and central nervous system as well as on other excitable tissues.

SUMMARY

1. The functional interrelationship between 5-hydroxytryptamine and melatonin, a substance originally isolated from the pineal body, was examined using the rat isolated duodenum preparation as a test object.

2. Inhibition of spontaneous contractions was observed following the administration of melatonin alone. The effect was dose-dependent, requiring about 1.25×10^{-9} moles/ml. for a very slight inhibition. There was no change of characteristic contraction frequency in the dose-range employed.

3. Melatonin suppressed the motile response of the duodenal segment to 5-hydroxytryptamine. The linear relationship found between the dose of 5-hydroxytryptamine and the concentration of melatonin required for 50% inhibition suggests a competition between the two substances.

The authors wish to thank Professor J. Magnes for his interest and constructive advice in this work. They are grateful to Dr Am. J. Meyer, from the Department of Organic Chemistry of the Hebrew University, who synthesized and kindly supplied us with melatonin for preliminary experiments. They are also indebted to Mrs J. Meron and Mr S. Ben Yonah for their valuable aid in preparing the manuscript and diagrams for publication. The work was communicated in part to the Israel Physiological and Pharmacological Society on April 21, 1964. A preliminary report appeared in the Israel *Journal of Experimental Medicine*, **11**, 207, 1964.

REFERENCES

AXELROD, J. & WEISSBACH, H. (1960). Enzymatic methylation of N-acetylserotonin to melatonin. *Science*, **131**, 1312.

AXELROD, J., MACLEAN, P. D., ALBERTS, R. W. & WEISSBACH, H. (1961). Regional distribution of methyl transferase enzymes in the nervous system and glandular tissues. In *Regional Neurochemistry*, ed. KETY, S. S. & ELKES, J., pp. 307–311. New York: Pergamon Press.

BASCHIERI, L., DE LUCA, F., CRAMAROSSA, L., DE MARTINO, C., OLIVARIO, A. & NEGRI, M. (1963). Modification of thyroid function by melatonin. *Experientia (Basel)*, **19**, 15.

BÜLBRING, E. (1961). The intrinsic nervous system of the intestine and the local effects of serotonin. In *Regional Neurochemistry*, ed. KETY, S. S. & ELKES, J., pp. 437–441. New York: Pergamon Press.

ERSPAMER, V. & ASERO, B. (1952). Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. *Nature (Lond.)*, **169**, 800–801.

FISKE, V. M., POUND, J. & PUTNAM, K. (1962). Effect of light on the weight of the pineal organ in the hypophysectomized, gonadectomized, adrenalectomized or thiouracil fed rats. *Endocrinology*, **71**, 130–133.

GIARMAN, N. J., FREEDMAN, D. X. & PICARD-AMI, L. (1960). Serotonin content of the pineal glands of man and monkey. *Nature (Lond.)*, **186**, 480–481.

GYERMEK, L. (1961). 5-Hydroxytryptamine antagonists. *Pharmacol. Rev.*, **13**, 339–439.

KAPPERS, J. A. (1962). Melatonin, a pineal compound. Preliminary investigations on its function in the rat. *Gen. comp. Endocr.*, **2**, 610–611.

KOPIN, I. J., PARE, C. M. B., AXELROD, J. & WEISSBACH, H. (1961). Fate of melatonin in animals. *J. biol. Chem.*, **236**, 3072–3075.

LERNER, A. B., CASE, J. D., MORI, W. & WRIGHT, M. R. (1959). Melatonin in peripheral nerve. *Nature (Lond.)*, **183**, 1821.

LERNER, A. B., CASE, J. D. & TAKAHASHI, Y. (1960). Isolation of melatonin and 5-methoxy-3-acetic acid from bovine pineal glands. *J. biol. Chem.*, **235**, 1992–1997.

LERNER, A. B., CASE, J. D., TAKAHASHI, Y., LEE, T. H. & MORI, W. (1958). Isolation of melatonin, the pineal gland factor that lightens melanocytes. *J. Amer. chem. Soc.*, **80**, 2587.

QUAY, W. B. (1961). Reduction of mammalian pineal weight and lipid during continuous light. *Gen. comp. Endocr.*, **1**, 211–217.

QUAY, W. B. (1963). Cytologic and metabolic parameters of pineal inhibition by continuous light in the rat. *Z. Zellforsch.*, **60**, 479–490.

QUAY, W. B. & HALEVY, A. (1962). Metabolic and cytologic evidence of pineal inhibition by continuous light. *Amer. Zool.*, **2**, 550.

ROTH, W., WURTMAN, R. J. & ALTSCHULE, M. D. (1962). Morphological changes in the pineal parenchymal cells of rats exposed to continuous light or darkness. *Endocrinology*, **71**, 888–892.

UMBREIT, W. W., BURRIS, R. H. & STAUFFER, J. F. (1957). *Manometric Techniques*, p. 149. Minneapolis: Burgess Publishing Co.

WEISSBACH, H., REDFIELD, B. G. & AXELROD, J. (1960). Biosynthesis of melatonin: enzymatic conversion of serotonin to N-acetylserotonin. *Biochim. biophys. Acta (Amst.)*, **43**, 352–353.

WURTMAN, R. J., AXELROD, J. & CHU, E. W. (1963). Melatonin, a pineal substance: effect on the rat ovary. *Science*, **141**, 277.

WURTMAN, R. J., AXELROD, J. & FISCHER, J. E. (1964). Melatonin synthesis in the pineal gland: effect of light mediated by the sympathetic system. *Science*, **143**, 1328–1329.

WURTMAN, R. J., AXELROD, J. & POTTER, L. T. (1964). The uptake of H^3-melatonin in endocrine and nervous tissue and the effect of constant light exposure. *J. Pharmacol. exp. Ther.*, **143**, 314–318.

WURTMAN, R. J., ROTH, W., ALTSCHULE, M. D. & WURTMAN, J. J. (1961). Interactions of the pineal and exposure to continuous light on organ weights of female rats. *Acta endocr. (Kbh.)*, **36**, 617–624.