Effect of sulpiride and hypothalamic extracts on prolactin release by rat pituitaries in vitro

Group and treatment [a]	Dose	Prolactin concentration [b]
1 Saline	—	217.6 ± 25.2 [c]
2 Sulpiride	1 mg	272.2 ± 31.0
3 Sulpiride	0.05 mg	352.0 ± 59.4 [d]
4 Rat hypothalamic extract	1.5 hypothalami	120.0 ± 4.4
5 Rat hypothalamic extract plus sulpiride	1.5 hypothalami + 1 mg	221.0 ± 41.2
6 Rat hypothalamic extract plus sulpiride	1.5 hypothalami + 0.05 mg	209.6 ± 36.0

[a] Three beakers containing 5 pituitary halves per each treatment were used. [b] Expressed as ng of NIAMDD - rat prolactin-RP, 1 mg of pituitary weight/ml of medium. [c] The variance analysis showed statistical significance of the differences among groups ($p < 0.05$). [d] Group 3 vs Group 1: $p < 0.05$ (Duncan's new multiple range test).

significance of the differences among groups was tested by means of the analysis of variance and the Duncan's new multiple range test [5].

Results. The analysis of variance showed that there is a significant difference in the concentration of prolactin in the media from the 6 different treatments investigated ($p < 0.05$). The Table shows that the media to which sulpiride was added, contained significantly higher concentrations than the control media where saline was added. The addition of rat hypothalamic extracts inhibited the release of prolactin. On the other hand, the media where hypothalamic extracts plus sulpiride were added contained higher concentrations of prolactin than those where only hypothalamic extracts were added. Due to the rather high dispersion of these values however, the Duncan test did not show the presence of statistical significance among them.

Discussion. It is a well known fact that the anterior pituitary gland is under hypothalamic control[6]. In the case of prolactin, this influence is mainly inhibitory[7]. When the anterior pituitary gland is removed from its normal location in the pituitary fossa and incubated in vitro, it releases increasing amounts of prolactin[8]. In our experiment, it seems evident that in such a circumstance sulpiride was able further to stimulate the prolactin release. Paradoxically, the lower dose used seemed to be more effective than the higher one in stimulating prolactin release. On the other hand, it is evident that the addition of sulpiride simultaneously with rat hypothalamic extracts inhibited to some extent the supressive effect of the prolactin release-inhibiting factor (PIF) contained in them on prolactin release. It thus seems evident that the action of sulpiride is exerted, at least in part, at the pituitary level. The results of this investigation do not exclude, however, the possibility that some of the effects of sulpiride reported in vivo could have been exerted also at the hypothalamic level[9].

Résumé. La sulpiride stimule la libération de la prolactine de l'hypophise après 4 h d'incubation. L'addition d'extraits hypothalamiques l'inhibe, mais la sulpiride ajoutée en même temps aux extraits hypothalamiques permet l'inversion de cet effet d'inhibition. Il semble evident que la sulpiride peut modifier la libération de la prolactine en agissant directement sur l'hypophise du rat, in vitro.

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Stimulation and Suppression of Somatomedin Activity by Serotonin and Melatonin

The pineal gland hormone, melatonin, and its indoleamine precursor, serotonin, were recently shown to exert opposing effects on the secretion of growth hormone in both the rat[1,2] and in man[3]. These effects of melatonin and serotonin were proposed as one way by which the pineal might regulate growth[2]. The present study was undertaken to determine whether the actions of these two indoles might extend to regulation of growth hormone action as well as its release from the pituitary gland.

Growth hormone is believed to exert its effects on skeletal growth via an intermediary substance which was originally called 'sulphation factor'[4]. More recently the general term 'somatomedin' was proposed[5] to include those substances having growth promoting activity

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Experiment No.	Test substance	Concentration of test substance relative to reference serum	Somatomedin ± SEM (U/ml)		Significance
1–13	Normal serum	—	0.952 ± 0.029		—
14–17	Serotonin + reference serum	1–10 picomole/µl	1.265 ± 0.073		$p < 0.005$
18	Melatonin + reference serum	1 picomole/µl	0.819		
19	Melatonin + reference serum	1 picomole/µl	0.800	0.652 ± 0.091	$p < 0.005$
20	Melatonin + reference serum	1 nanomole/µl	0.622		
21	Melatonin + reference serum	1 nanomole/µl	0.366		

which were previously given terms relating to a specific activity such as cartilage sulphation[4] or thymidine incorporation into DNA[6]. Partial characterization of somatomedin suggests that it is a polypeptide of molecular weight less than 10,000[7].

Available evidence suggests that serotonin enhances somatomedin activity. Serotonin has been shown to cause a significant increase in DNA synthesis in guinea-pig skin slices[8]; it increases the rate of mitosis of cultured fibroblast cells[9] as well as that of various rat and mouse tissues including ascites tumour cells[10]. Furthermore, there is evidence to suggest that serotonin is released and/or synthesized peripherally under the stimulus of growth hormone. LABORIT et al.[11] found increased amounts of the serotonin metabolite, 5-hydroxyindoleacetic acid, in the urine of rabbits after they were injected with growth hormone. SIREK et al.[12] showed that serotonin is released into the pancreaticoduodenal circulation of dogs following growth hormone administration. In man a similar situation seems to exist as it was recently reported[13] that following resection of pituitary tumours in acromegalic subjects, previously elevated serum serotonin levels fell to normal in parallel with the fall in serum growth hormone.

Little is known of the actions of melatonin in the peripheral circulation but if it does act as a competitive inhibitor of serotonin at its receptors according to our hypothesis[2], then it should interfere with any stimulatory effect serotonin may have on somatomedin activity. In support of an inhibitory action of melatonin, we have found that it reduces the in vivo incorporation of $^{35}SO_4^=$ into rat cartilage[14]. BOUCEK and ALVAREZ[9] reported that 5-methoxytryptamine reduces mitosis in cultured fibroblast cells by 50%. 5-Methoxytryptamine is a direct precursor of melatonin and both methoxy indoles are present in the pineal gland to about the same extent[15]. We have previously suggested[2] that the important structural feature of melatonin which results in it having actions opposing those of serotonin is the 5-methoxy group and hence 5-methoxytryptamine would be expected to exert a similar, and perhaps greater, ability.

In this study, somatomedin activity was determined by measuring the stimulation of $^{35}SO_4^=$ uptake into costal cartilage segments from intact weanling rats[16]. Serum from a normal female was used as a reference standard and it was arbitrarily assigned a somatomedin level of 1 unit/ml. In a series of 4-point assay systems serotonin or melatonin was added to the assay system at 2 dose levels of reference serum and the uptake of $^{35}SO_4^=$ was compared with that of the system containing the same dose levels of reference serum alone. The results are shown in the Table. p-Values were calculated against somatomedin levels obtained for serum samples from 13 normal subjects. The concentration of serotonin used was based on that reported to increase mitosis in cultured fibroblasts[9].

At the concentration tested (1–10 picomole/µl), the addition of serotonin to reference serum caused a highly significant increase in the uptake of labelled sulphate by cartilage. Conversley, melatonin highly significantly inhibited labelled sulphate incorporation. This effect of melatonin seemed to be dose related, being most effective at the higher concentration but to establish this, a more complete examination is required. For the purpose of this study, the results obtained at the different concentrations of melatonin were combined and show a mean suppression of somatomedin activity of approximately 35%.

The results presented demonstrate that serotonin and melatonin have highly significant, but opposite, effects on somatomedin activity. This finding shows that the influence of these indole derivatives on growth involves not only the central control of growth hormone release from the pituitary[2] but also the peripheral control of the growth promoting activity of somatomedin.

Résumé. La sérotonine et sa dérivée, la mélatonine, hormones de la glande pinéale, ont les effets très significatifs mais opposés sur l'activité de la somatomédine. L'addition de la sérotonine au sérum normal a fait augmenter l'activité de la somatomédine de 0.952 ± 0.029 unités/ml à 1.265 ± 0.073 unités/ml ($p < 0.005$) tandis que l'addition de la mélatonine l'a abaissée à 0.652 ± 0.091 ($p < 0.005$).

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