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Pineal 'synaptic' ribbon numbers and melatonin synthesis of rat are resistant to guanethidine sympathectomy

S. Reuss and T. Kreis

Department of Anatomy, Johannes Gutenberg-University, Saarstr. 19-21, D-55099 Mainz (Germany)
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Abstract. Chemical sympathectomy is widely used to study the impact of the noradrenergic system on neuronal and neuroendocrine circuits. We tested the effects of intraperitoneal injections of guanethidine, an adrenergic neuron blocking agent, on selected functional parameters of the rat pineal gland which are known to be under sympathetic influence. The reliability of the method was demonstrated by the clear enophthalmus developed by experimental animals. However, neither the numbers of 'synaptic' ribbons nor melatonin synthesis differed between treated and control rats, both parameters exhibiting the nocturnal increase seen in intact animals. These results are in striking contrast to those obtained upon chemical sympathectomy with 6-hydroxydopamine or surgical superior cervical ganglionectomy. We conclude that guanethidine is not capable of sufficiently removing noradrenergic influence from the rat pineal gland, and that this substance is thus inferior to other experimental methods of sympathectomy.
Key words. Guanethidine; melatonin; pineal; rat; ribbons; sympathectomy.

The rodent pineal gland is richly innervated by sympathetic fibers originating in the superior cervical ganglia (SCG)[1,2]. These are connected via the spinal cord to the circadian oscillator in the hypothalamic suprachiasmatic nucleus and regulate, by the differential release of noradrenaline (NA), the distinct 24-h rhythm of pineal function. Melatonin synthesis[3] is relatively low during the daytime and increases at night. A considerable number of studies have utilized different methods of sympathetic denervation of the gland to investigate the impact of noradrenergic innervation on pineal functional parameters. For example, acute surgical superior cervical ganglionectomy (SCGX) was found to disrupt the nocturnal increase of melatonin synthesis[4] and to decrease the number of 'synaptic' ribbons[5] (SR), rodlike organelles surrounded by clear vesicles probably acting as functional links between adjacent cells. SR numbers are in the range of several hundreds per pinealocyte and display a circadian rhythm in pineal morphology with high amounts at night[6,7]. Chronic SCGX or chemical sympathectomy of newborn rats using the false neurotransmitter 6-hydroxydopamine (6-OHDA), however, increased SR numbers[5,8]. We conducted the present study to determine the effects of guanethidine sympathectomy, which suppresses postganglionic adrenergic nerve function by presynaptic inhibition of neurotransmitter release, a rapid method widely used for chemical sympathectomy (e.g., ref. 9). We determine pineal SR numbers during the day and at night in treated, sham-treated and control rats. In the same pineal glands we also studied the activity of the melatonin-forming enzyme, serotonin N-acetyltransferase (NAT), which has not yet been investigated following chemical sympathectomy.

Materials and methods

Thirty-two adult male Sprague-Dawley rats (200–220 g b.wt.), obtained from the Zentralinstitut für Versuchstierzucht, Hannover, Germany, were maintained with food and water ad libitum in a light-controlled (LD 12:12), temperature-regulated room. For chemical sympathectomy[9], 12 of them were given daily injections of guanethidine (Sigma, 50 mg/kg) dissolved in physiological saline on experimental days 1–5. Ten control animals received vehicle injections only. Ten additional animals served as untreated controls. On experimental day 7, animals were killed by decapitation in the middle of the light or dark period. Pineal glands were quickly removed and cut into halves which were either frozen in liquid nitrogen for biochemical analysis or fixed according to Karnovsky[10] for 20 h and processed for routine electron microscopy as described in detail previously[8]. Examination of ultrathin sections was performed in such a way that the investigator was unaware from which group of animals the material was taken. For the quantitative assessments of SR, pineal tissue covering 5 adjacent grid holes was carefully scanned at $\times 20{,}000$ magnification, and the total number of SR was counted (typical examples of these structures were depicted in ref. 8). SR numbers were calculated per 20,000 μm^2 corresponding to 1 unit area (UA).

For biochemical analysis, tissue specimens were homogenized in an ice-cold tryptamine solution (2.1 mM

tryptamine in 50 mM phosphate buffer, pH 6.5) within 24 h and the activity of pineal N-acetyltransferase (NAT; EC 2.3.1.5) was determined[11]. Protein concentrations of the samples were determined according to Lowry et al.[12] with bovine serum albumin as a standard. The Wilcoxon-Mann-Whitney U-test was used for statistical evaluation of differences in SR number between animal groups. Statistical treatment of the NAT data involved an analysis of variance followed by Duncan's multiple range comparison of means.

Results

Guanethidine-treated rats, but not sham-treated or control animals, exhibited a distinct enophthalmus indicating that the procedure of chemical sympathectomy was successful. No significant differences with regard to the tested pineal parameters were observed between treated, sham-treated or untreated animals (table). The N-acetyltransferase activity was undetectable at daytime and was high in the middle of the dark period. In addition, mean numbers of 'synaptic' ribbons were similar in all groups, with the nocturnal levels elevated by approximately fifty percent.

Discussion

The findings of the present study reveal that selected morphological and metabolic pineal parameters of adult rats are not sensitive to guanethidine treatment. SR numbers were similar in each comparable group, in contrast to previous findings of augmented SR numbers in animals sympathectomized by 6-hydroxydopamine (6-OHDA)[8] or surgical superior cervical ganglionectomy (SCGX)[5]. In addition, we observed a similar nocturnal increase of NAT activity, typical for the intact mammalian pineal gland, in animals of treated and control groups, whereas complete surgical removal of the sympathetic input to the pineal of rats abolished the nocturnal peak of melatonin synthesis[4]. Thus, the conclusion drawn from our study is that guanethidine is not capable of sufficiently removing noradrenergic influence from the rat pineal gland in experimental situations, although the distinct enophthalmus observed in treated animals indicated that the procedure of chemical sympathectomy was successful.

Table. Numbers of pineal 'synaptic' ribbons (SR) and activity of N-acetyltransferase (NAT; nmol/h · mg protein) by day or night in pineal glands from three rat groups (control, vehicle-injected, guanethidine-treatment).

	Control	Vehicle	Guanethidine
Day			
SR	36.4 ± 1.9	38.8 ± 4.5	39.7 ± 4.1
NAT	n.d.	n.d.	n.d.
Night			
SR	51.6 ± 7.0	53.6 ± 3.0	55.0 ± 3.3
NAT	84 ± 8.4	81 ± 12.9	77 ± 12.4

Statistical differences were observed between day and night levels of SR ($p < 0.05$) in each group, but not between treated and control groups. n.d. = not detectable.

The present results, on the other hand, may shed further light on the relative importance of the noradrenergic innervation for pineal function. There is evidence that the central (nonsympathetic) pineal innervation via its stalk[13,14] is involved in the numerical upregulation of SR at night since transection of the pineal stalk in rat resulted in diminished nocturnal SR numbers (Kreis and Reuss, unpubl. results). Whether this neural pathway plays a role in melatonin synthesis as well is, however, as yet unknown. Regardless of its origin, a non-adrenergic input to the gland is provided by various neuropeptide-containing fibers in the pineal. Arginine-vasopressin, vasoactive intestinal polypeptide and neuropeptide Y, for example, are thought to be involved in the regulation of pineal metabolism[15–18], somatostatin has been shown to increase pineal SR numbers in vitro[18], and there is also evidence for the influence of serotonin[20,21] and corticosterone[22] on pineal function.

Although the reasons for pineal resistance to guanethidine sympathectomy are unclear we suggest that in experimental studies in which sympathectomy of the pineal of rat (and probably other laboratory rodents) is to be achieved, other methods such as surgical ganglionectomy or the use of 6-hydroxydopamine should be used in preference to guanethidine treatment.

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