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Reduction of regional cerebral blood flow by melatonin in young rats

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MELATONIN (10 ng) was subcutaneously administered to 14-day-old Sprague–Dawley rats. Regional blood flow (rCBF) was measured in 22 anatomically defined structures 20 min later using iodo[^{14}C]antipyrine and quantitative autoradiography. rCBF was markedly reduced in the cerebral areas supplied by circle of Willis and the basilar arteries. Melatonin also significantly decreased blood flow to choroid plexuses. These findings suggest that circulating melatonin may contribute to regulation of cerebral blood flow and brain fluid balance.

Key words: Melatonin; Regional cerebral blood flow; Choroid plexus; Young rats; Receptors

Introduction

Cerebral blood flow is influenced by variations in systemic blood pressure.[1] Many substances can regulate perfusion pressure, including melatonin, a 5-methoxyindole synthesized mainly in the pineal gland, that can induce hypotension in rats[2] and humans.[3] Although melatonin receptors have been characterized in rat area postrema[4,5] and cerebral arteries[6] a direct effect of melatonin on cerebral blood flow has not been demonstrated. The experiments reported in this study examined the effects of melatonin, administered at physiological doses, on the cerebral blood flow in young rats.

Materials and Methods

Immature rats (7 days old) were purchased from Charles River (Como, Italy) and reared with their mothers for 7 days under a 12:12 h light:dark cycle (lights on at 07.00 h).

Regional cerebral blood flow (rCBF) was determined by the iodo[^{14}C]antipyrine method as described by Sakurada *et al*,[7] modified for very small animals.[8,9] Rat pups (25–30 g) received a s.c. injection of 0.1 ml saline (control animals) or melatonin (341 ng in 0.1 ml saline; ethanol < 0.001%) 3 h after lights on. The injection was made on the back, between the shoulder blades, and if bleeding or leakage from the injection site occurred the pup was not used in the experiment. After 15 min control and melatonin-treated animals received a s.c. injection of 5 μCi 4-iodo-(N-methyl-^{14}C)-antipyrine (New England Nuclear, Boston, MA). Five minutes later the pups were decapitated and trunk blood collected in heparinized tubes. Ten millilitres of blood were added to liquid scintillation cocktail in a vial and counted to determine blood tracer concentrations (d.p.m. ml^{-1}). The brains were removed and immediately frozen on dry ice (−70°C). Horizontal sections (20 μm thick) were obtained in a cryostat kept at −16°C.

Brain sections were mounted on slides, dried at 55°C and exposed in a light-proof cassette with ^{14}C radioactive standards (ARC, St Louis, MO). Autoradiograms were then processed for quantitative autoradiography and rCBF for specific regions of the rat brain was calculated according to the modification of Fick equation by Kety.[10] Statistical comparison was undertaken using Dunnett's test.

The presence of 2-[^{125}I]iodomelatonin binding sites in rat basilar artery and choroid plexus was determined in coronal sections from rat brain stem containing the plexus of the fourth ventricle, the area postrema and the basilar artery. Slide-mounted sections were incubated with 2-[^{125}I]iodomelatonin (S.A. 1800–2000, Amersham, UK) as described previously.[11] Non-specific binding was determined, in along side sections, in presence of excess unlabelled 2-iodomelatonin (RBI, Natick, MA).

Results

The data shown in Table 1 and Figure 1 represent rCBF values for 22 anatomically distinct structures of the immature rat brain. The lowest values observed were in the subcortical and pontine white matter. The highest value was exhibited by choroid plexuses. Melatonin, at physiological doses, significantly reduced rCBF to the cerebral hemispheres, diencephalic and brain stem gray matter. Blood flow to the cerebral cortex (cingulate gyrus, frontal and parietal cortex), hippocampus (stratum pyramidale and granulosum dentatum), thalamus, anterior and posterior colliculi and cerebellar vermis was reduced by approximately 60% ($p < 0.01$). This decrease was less pronounced ($p < 0.05$) in occipital and temporal cortex, hypothala-

Table 1. Regional cerebral blood flow in immature rats after melatonin treatment. Values (ml g^{-1} tissues min^{-1}) represent the mean ± s.e.m.

	Saline ($n = 4$)	Melatonin ($n = 4$)
Frontal cortex	41 ± 4	16 ± 4**
Cingulate gyrus	44 ± 5	19 ± 4**
Parietal cortex	42 ± 5	17 ± 4**
Occipital cortex	42 ± 4	18 ± 5*
Temporal cortex	38 ± 6	18 ± 4*
Pyriform cortex	37 ± 6	18 ± 4
Neostriatum	38 ± 5	18 ± 4
Hippocampal stratum pyramidale	50 ± 6	20 ± 5**
Hippocampal stratum granulosum	47 ± 6	20 ± 5**
Hippocampal stratum molecularis	37 ± 6	19 ± 5
Hypothalamus	40 ± 6	18 ± 5*
Thalamus	43 ± 6	21 ± 5**
Anterior colliculi	47 ± 6	19 ± 4**
Posterior colliculi	51 ± 5	21 ± 6**
Pontine nuclei	46 ± 5	22 ± 6*
Vermis cerebellum	50 ± 5	21 ± 6**
Cerebellar hemispheres	42 ± 5	19 ± 5*
Choroid plexuses	65 ± 10	24 ± 6**
White matter	29 ± 5	19 ± 4

*$p < 0.05$; **$p < 0.01$ (Dunnett)

mus, pontine gray matter and lateral cerebellar hemispheres. Moreover, melatonin also produced a marked reduction of rCBF to the choroid plexuses. Melatonin did not affect rCBF to a statistically significant extent in the pyriform cortex, neostriatum and cerebral white matter. The decrease in rCBF in melatonin-treated pups was not associated with any detectable alterations in the histological structure of nervous tissue supplied (data not shown).

Autoradiographic studies established the presence of specific 2-[^{125}I]iodomelatonin binding in area postrema, the choroid plexuses and basilar artery of young rats (Fig. 2), as well as in the intracerebral branches of the internal carotid arteries (data not shown).

Discussion

2-[^{125}I]Iodomelatonin, a radiolabelled melatonin agonist used to characterize melatonin receptors,[14] specifically binds to rat cerebral arteries forming the circle of Willis.[6] The demonstration that melatonin receptors in rat cerebral arteries are linked to the adenylate cyclase second messenger system[15] and the presence of a similar distribution of melatonin-binding sites in primate cerebral arteries[16] prompted us to study the effects of melatonin on cerebral blood flow. The higher density of melatonin receptors in the anterior cerebral arteries of young rats[17] suggested performing experiments on cerebral blood flow in immature animals.

The results obtained in this study confirm the reproducibility of the technique used by Lyons[8] and Vannucci.[9] The values of rCBF in our control pups are very similar to those obtained in their studies on immature rats. This approach bypasses the known effects of anaesthesia and surgery on cerebral blood flow and avoids immobilization stress interfering with the parameters studied during the experiment.[1] Although the differences were not statistically significant, the mean blood flow to midbrain, pons and cerebellum was higher than that to cerebral cortex and to diencephalic structures in controls, as has been demonstrated in perinatal animals of other species.[8,9,12,13] In our study we were not able to analyse the amount of melatonin in pup peripheral blood since the collected trunk blood was needed to determine the quantity of iodo[^{14}C]antipyrine. However, the calculated total amount of melatonin was about 1 ng ml^{-1} blood. The peak physiological melatonin concentration during the night is (100–300 pg ml^{-1}).[18] Thus, the animals received

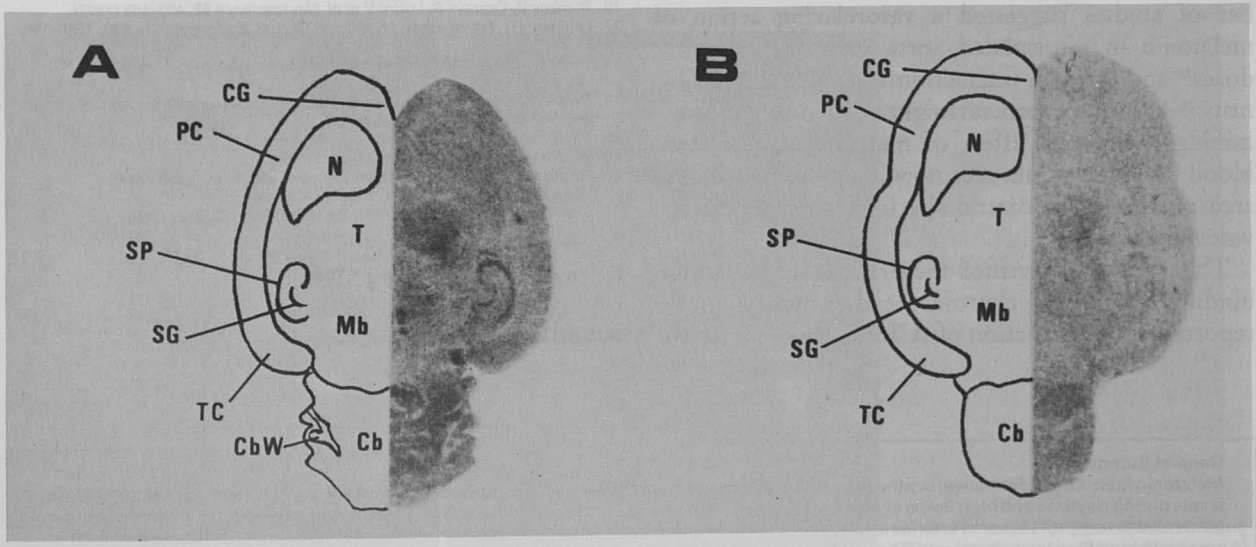

FIG. 1. Iodo[^{14}C]antipyrine-generated autoradiograms representing the rCBF in horizontal sections of rat brains obtained after saline (**A**) or melatonin administration (10 ng; **B**). Cb, cerebellum; CbW, cerebellar white matter; GC, cingulate gyrus; Mb, midbrain; N, neostriatum; PC, parietal cortex; SG, hippocampal stratum granulosum; SP, hippocampal stratum pyramidale; T, thalamus; TC, temporal cortex.

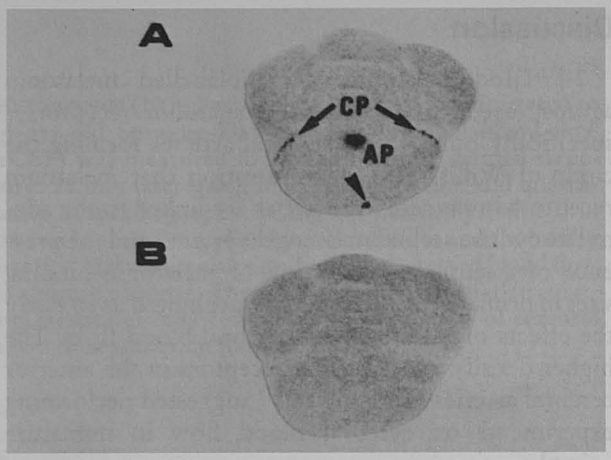

FIG. 2. Autoradiograms from coronal sections through the brain stem, generated with 2-[^{125}I]iodomelatonin, showing melatonin binding sites in newborn rat area postrema (AP), choroid plexus of the IVth ventricle (CP) and the basilar artery (arrowhead). (**A**) Specific binding. (**B**) Non-specific binding determined in presence of excess unlabelled 2-iodomelatonin.

acutely a physiological dose of melatonin. Melatonin may have important physiological or pathophysiological effects on cerebral vasculature.

This result extends further the reports on a vasoconstrictive effect of melatonin in young rat caudal artery[19] and data regarding the physiological effects of melatonin on the second messenger mechanism regulated by adenylate cyclase. Melatonin (10 nM) was reported to counteract the increase of cAMP induced by forskolin, a vasodilatory agent.[16] Alternatively, the major vasoconstructor action of melatonin may occur not at the melatonin receptor on the vascular smooth muscle cells, but secondary to the hypotensive effect exerted at another site. Evidence of the hypotensive effect of melatonin has been obtained through studies in patients with essential hypertension[3] and in experiments with spontaneously hypertensive rats.[2] A number of studies suggested a vasorelaxing action of melatonin in rat isolated aorta at pharmacological doses[20] and through interaction with adrenergic agonists.[21] A third mechanism might be involved in determining the overall effect of melatonin on cerebral blood flow: melatonin receptors are present in the rat area postrema,[4,5] a central site implicated in cardiovascular control.[22]

This study also verified the presence of melatonin binding sites in rat choroid plexuses, as previously reported.[23] The reduction of rCBF in these structures suggests that circulating melatonin may contribute to regulation of fluid balance and production of cerebrospinal fluid. The demonstration of the presence of melatonin binding sites in a portion of the basilar artery which was not examined before broadens the knowledge on the distribution of melatonin receptors in the basal brain arteries. This may explain the reduction of cerebral blood flow in brain stem areas vascularized by the basilar artery and its branches.

The effect of melatonin on cerebral blood flow may be coupled to a decrease of cerebral metabolism. A reduction of glucose uptake has been reported in adult rats at physiological doses.[24]

Conclusion

This study is, to our knowledge, the first report on the effects of a physiological dose of melatonin on cerebral blood flow. The investigation performed in immature rats, as laboratory models, adds new information not only on central melatonin effects but also on vascular physiology of newborn mammals.

References

1. Edvinsson L, MacKenzie ET and McCulloch J. *Cerebral Blood Flow and Metabolism*. New York: Raven Press, 1993.
2. Kawashima K, Miwa Y, Fujimoto K *et al. Clin Exp Hypertens [A]* **9**, 1121–1131 (1987).
3. Birau N, Peterssen U, Meyer C *et al. IRCS Med Sci* **9**, 906 (1981).
4. Weaver DR, Rivkees SA and Reppert SA. *J Neurosci* **9**, 2581–2590 (1989).
5. Laitinen JT, Flugge G and Saavedra JM. *Neuroendocrinology* **51**, 619–624 (1990).
6. Viswanathan M, Laitinen JT and Saavedra JM. *Proc Natl Acad Sci USA* **87**, 6200–6203 (1990).
7. Sakurada O, Kennedy C, Jehle J *et al. Am J Physiol* **234**, H59–H66 (1978).
8. Lyons DT, Vasta F and Vannucci RC. *Pediatr Res* **21**, 471–476 (1987).
9. Vannucci RC, Lyons DT and Vasta F. *Stroke* **19**, 245–250 (1988).
10. Kety SS. *Methods Med Res* **8**, 228–236 (1960).
11. Stankov B, Cozzi B, Lucini V *et al. Neurosci Lett* **133**, 68–72 (1991).
12. Cavazzuti M and Duffy TE. *Ann Neurol* **11**, 247–257 (1982).
13. Rosenberg AA, Jones MD, Traystman RJ *et al. Am J Physiol* **242**, H862–H866 (1982).
14. Morgan PJ, Barrett P, Howell HE *et al. Neurochem Int* **24**, 101–146 (1994).
15. Capsoni S, Viswanathan M, De Oliveira AM *et al. Endocrinology* **135**, 373–378 (1994).
16. Stankov B, Capsoni S, Lucini V *et al. Neuroscience* **52**, 459–468 (1993).
17. Laitinen JT, Viswanathan M, Vakkuri O *et al. Endocrinology* **130**, 1896–1902 (1992).
18. Lewy AJ, Tetsud M, Markey SP *et al. J Clin Endocrinol Metab* **50**, 204–205 (1979).
19. Evans BK, Mason R and Wilson VG. *Naunyn-Schmiedeberg's Arch Pharmacol* **346**, 362–365 (1992).
20. Satake N, Oe H and Shibata S. *Gen Pharmacol* **22**, 1127–1133 (1991).
21. Weekley LB. *J Pineal Res* **11**, 28–34 (1991).
22. Ferguson AV and Marcus P. *Am J Physiol* **255**, R855–R860 (1988).
23. Vanecek J. *J Neurochem* **51**, 1436–1440 (1988).
24. Vitte PA, Brun J, Lestage P *et al. Brain Res* **489**, 273–282 (1989).

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General Summary
Melatonin is an indolic derivative, synthesized by the vertebrate pineal gland and having a well-defined cyclical circadian variation with low levels during daytime and high levels at night. Melatonin is known mainly for its effects on brain areas involved in circadian rhythms but it binds also to receptors in some arteries at the base of the brain. Melatonin possesses general hypotensive effects and induces a decrease in cerebral blood flow in neonatal rats. The effect can be mediated through interaction with specific receptors either in the arteries, or in the area postrema, a central site involved in cardiovascular control.