A comparative study of picomolar affinity 2-[\(^{125}\)I]iodomelatonin binding sites in the hearts of three salmonid species

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Abstract

The hearts of three cultured salmonid species, collected at either mid-light or mid-dark were studied for their binding to 2-[\(^{125}\)I]iodomelatonin, a specific melatonin agonist. The binding was saturable, reversible, and highly specific. The equilibrium dissociation constant (K\(_d\)) ranged from 30.1 ± 3.0 pmole \(1^{-1}\) in Arctic charr (Salvelinus alpinus) to 40.5 ± 2.3 pmole \(1^{-1}\) in rainbow trout (Oncorhynchus mykiss) indicating a high binding affinity. The maximum density of binding (B\(_{\text{max}}\)) was at the low femtomolar level of 0.57 to 0.87 fmole mg\(^{-1}\) protein. Higher B\(_{\text{max}}\) appeared to be demonstrated in the mid-light samples when compared to the mid-dark samples but the difference was not significant (\(p > 0.05\)). Competition study with various indoles showed the following order of potency: 2-iodomelatonin > melatonin > 6-chloromelatonin >> N-acetylserotonin >> serotonin. Guanosine 5'-O-(3-thiotriphosphate) (GTP\(_{\gamma}\)S) strongly inhibited the binding (IC\(_{50}\) = 0.66 \(\mu\)mole \(1^{-1}\)) in the rainbow trout heart, suggesting that these binding sites belong to the superfamily of G-protein linked receptors. Our results suggest the presence of melatonin receptors in the fish heart. In addition, there was no marked intraspecies differences in K\(_d\), B\(_{\text{max}}\) and specificity that could be correlated with the phylogeny or life history of the salmonid species.

Introduction

In fishes, as with other classes of vertebrates, melatonin is secreted by the pineal gland, with the highest secretory activity taking place during the dark phase (Pang 1985; Reiter 1991; Falcon \textit{et al}. 1992; Gern \textit{et al}. 1992). In addition, there is evidence in some fishes to suggest that some of the melatonin present in the blood may originate from the retina (Gern 1978). In those fishes examined to date, the secretion of melatonin is strongly photoperiod dependent (Gern \textit{et al}. 1978; Zachmann \textit{et al}. 1992), and there is convincing evidence to indicate that the hormone plays an important role in regulating seasonally-dependent events, such as the circannual reproductive activity. The manner in which the hormone affects the annual reproductive cycles of fishes is poorly understood, although in mammals a direct action of melatonin on the hypothalamic areas that regulate gonadotropin secretion, and on gonadotropin-secreting cells themselves is suspected (Reiter 1987; Zachmann \textit{et al}. 1992).

In some birds, mammals and fishes, the radio-
labelled ligand, 2-[¹²⁵]iodomelatonin (IM) has been used to identify tissues that specifically bind melatonin, and to characterize the nature of the putative melatonin receptors in those tissues. IM binding has been reported in several tissues of birds and mammals, including the brain, immune system, lung and heart (Stankov and Reiter 1990; Yuan and Pang 1990; Pang et al. 1993a; Gauer et al. 1992). In fishes, IM binding studies have been limited to whole brain preparations (Ekstrom and Vanacek 1992; Pang et al. 1994a). In this study, we explored the possibility of IM binding to heart tissue of fishes; the study was prompted by the recent finding of IM binding to heart tissue of birds (Pang et al. 1993a) and the long recognised action of melatonin on blood pressure changes in mammals (Holmes and Sugden 1975). The purpose of the study was to evaluate whether IM binding to heart tissue membranes was present in fishes, and if so, to characterize the affinity, concentration and pharmacology of the binding sites.

The effects of guanosine 5'-O-(3-thiotriphosphate) (GTPγS) on IM binding was also tested. This non-hydrolyzable guanine nucleotide was used instead of guanosine triphosphate (GTP) because the latter is easily hydrolysed in the signal transduction process and the transient effect is difficult to test. The inhibitory effect of this guanine nucleotide has been used as an evidence for receptor coupling to the G-proteins (Laitinen 1990). Therefore it was used in this study to test for its inhibitory effect in an attempt to further characterize this membrane binding site.

For the study, we chose three salmonids species from three different genera: Atlantic salmon (Salmo salar) [2 + year class; n = 12; body weight 418 ± 26 g (mean ± SEM)], Arctic charr (Salvelinus alpinus) [3 + year class; n = 12; body weight 776 ± 44 g] and rainbow trout (Oncorhynchus mykiss) [2 + year class; n = 12; body weight 660 ± 47 g] of both sexes were obtained from stocks maintained at the Alma Research Station, University of Guelph. All samples were taken in November, 1992, either at the middle of the light period (ML: 10:00-12:00h) or the middle of the dark period (MD: 23:30-00:30h).

The Atlantic salmon were maintained under a natural photoperiod (approximately 10:14 LD in November) in circular outside concrete ponds (12 m in diameter) in constantly running and aerated well water and fed commercial salmonid diets ad libitum by means of demand feeders.

The Arctic charr and rainbow trout were maintained in fibreglass aquaria (4 m in diameter) that were located inside aquarium buildings, under a light regime that approximated natural photoperiod. They were maintained in constantly running and aerated well water, and were fed a commercial salmonid diet each day by means of an automated feeder that delivered food over a 5h period beginning at 09:00h (Arctic charr) or by handfeeding twice daily to satiety (rainbow trout).

Collection of samples

The fish were killed by a blow to the head and the heart tissue rapidly removed and frozen on dry ice. The tissues were subsequently stored frozen at −70°C until the melatonin binding assays were