J. Pharm. Pharmacol. 1989, 41: 356–357
Communicated September 29, 1988

2-[^{125}I]Iodomelatonin binding sites in hamster and chick exhibit differential sensitivity to prazosin

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Abstract—Binding of 2-[^{125}I]iodomelatonin to putative receptors for melatonin has been well documented in chicken and hamster tissues. Differences in the potency of *N*-acetyl-5-hydroxytryptamine in these tissues suggested distinct central and peripheral binding sites. Prazosin was found to be much more potent ($K_i = 6{\cdot}5$ nM) in hamster hypothalamus than in chick retina ($K_i = 740$ nM). *N*-acetyl-5-HT also was more potent in hamster hypothalamus ($K_i = 14$ nM) than in chick retina ($K_i = 1050$ nM). A comparison of hamster hypothalamus to chick forebrain revealed a similar difference in potency for prazosin. In light of these findings, the dissimilarity between the potencies of *N*-acetyl-5-HT and prazosin in hamster and chick would appear to be due to a species difference as the central and peripheral sites seem alike within either species.

The pineal hormone melatonin has long been known to be involved in the regulation of neuroendocrine systems in many photoperiodic animals such as the hamster (Reiter et al 1976). Only recently has the use of the high specific activity radioligand 2-[^{125}I]iodomelatonin allowed the pharmacological identification of putative melatonin receptor binding sites in the chicken (Dubocovich & Takahashi 1987) and the hamster (Duncan et al 1986, 1988; Niles et al 1987). Aside from indoles structurally related to melatonin, only the drug prazosin had significant affinity for these sites in hamster brain tissues (Niles et al 1987). This work was undertaken to compare the prazosin sensitivity of this site in hamster tissues to that of chick tissues.

Materials and methods

2-[^{125}I]iodomelatonin was prepared as previously described (Niles et al 1987). Melatonin, *N*-acetyl-5-hydroxytryptamine and prazosin were purchased from Sigma Chemical Co. 6-Chloro-melatonin was a gift from Lilly Research Laboratories. All other reagents were obtained from commercial sources. Male (2–3 month old) Syrian hamsters (Charles River) were individually housed and maintained on a 14 h light:10 h dark cycle with free access to food (Purina Rat Chow) and water. Male leghorn chicks were decapitated at five days of age. Tissues were dissected on ice by the method of Glowinsky & Iversen (1966) and used fresh, or frozen at −60°C, thawed and used. Chick retinae were homogenized in ice-cold 0·32 M sucrose and centrifuged 10 min at 1000 *g* to remove most of the black epithelial pigment together with the nuclear fraction. The resultant supernatant was centrifuged 10 min at 48 000 *g*, the pellet resuspended in 50 mM Tris-HCl pH 7·4 (4°C) and washed twice. Tissue whole homogenates (chick forebrain) and crude synaptosomal membranes (hamster hypothalamus and testes) were prepared and used in competition and saturation experiments as previously described (Niles et al 1988). Competition data were analysed using the programme CDATA (EMF Software) and saturation data using the programme BDATA (EMF Software).

Results and discussion

Saturation binding studies (Table 1) indicate that sites of similar affinity for 2-[^{125}I]iodomelatonin exist in hamster hypothalamus, hamster testes, chick forebrain and chick retina. The highest density of sites occurs in the chick forebrain (170 fmol (mg protein)$^{-1}$) and lowest in the hamster testes (18 fmol (mg protein)$^{-1}$). The presence of these high affinity sites in the testes intimates that melatonin has direct effects in this tissue. This is in keeping with its known role of regulating the reproductive cycle of hamsters (Reiter et al 1976).

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Table 1. 2-[^{125}I]iodomelatonin binding in selected tissues. 2-[^{125}I]iodomelatonin was used in the concentration range 0·06–3·5 nM for saturation experiments. Means of triplicate values from one experiment are presented.

Tissue	K_d (nM)	B_{max} (fmol (mg protein)$^{-1}$)
Hamster hypothalamus	1·8	75
Hamster testes	1·1	18
Chick forebrain	1·6	170
Chick retina	0·69	66

Table 2. A pharmacological comparison of 2-[^{125}I]iodomelatonin binding sites in the hamster and chick.

	K_i (nM)[a]			
	Hamster		Chick	
	Hypothalamus	Testes	Forebrain	Retina
Melatonin	26	16	3·2	3·5
N-acetyl-5-HT	14	42	89	1050
Prazosin	6·5	30	510	740

[a] Calculated from the IC50 value (Cheng & Prusoff 1973). Means of triplicate values from one experiment are presented.

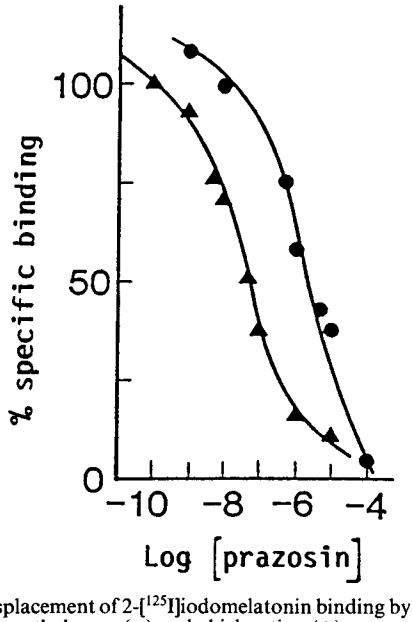

FIG. 1. Displacement of 2-[^{125}I]iodomelatonin binding by prazosin in hamster hypothalamus (▲) and chick retina (●).

2-[^{125}I]iodomelatonin binding sites in both chick forebrain and retina exhibit a high affinity for melatonin and much lower affinity for *N*-acetyl-5-HT (Table 2). Melatonin and *N*-acetyl-5-HT have approximately equal affinities at this site in hamster hypothalamus and testes. Melatonin itself is 4–8 fold more potent in the chick than in the hamster, arguing a species difference between the sites. Prazosin has much higher affinity for this site in hamster tissues than in chick tissues (Table 2 and Fig. 1). While the central and peripheral 2-[^{125}I]iodomelatonin sites seem similar within the hamster or within the chick, a species difference exists between the hamster and chick. It remains to be seen if functional differences exist across species.

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J. Pharm. Pharmacol. 1989, 41: 357–359
Communicated July 27, 1988

A study of β-adrenoceptors in rat lung parenchymal strip

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Abstract—The aim of the present study was to characterize the β-adrenoceptor population in rat lung strip. For this purpose, Schild plots were obtained for the β-adrenoceptor antagonists atenolol (β_1-selective), butoxamine (β_2-selective) and propranolol (non-selective), using three different agonists: isoprenaline (non-selective), salbutamol (β_2-selective) and noradrenaline (β_1-selective). The slopes of these Schild plots were close to the theoretical value of unity, and pA_2 values determined with isoprenaline, salbutamol and noradrenaline as agonists were: for propranolol, 7·86 ± 0·22, 7·72 ± 0·15 and 7·89 ± 0·23; for atenolol, 5·19 ± 0·05, 5·33 ± 0·07 and 5·47 ± 0·22 and for butoxamine, 6·31 ± 0·11, 6·34 ± 0·03 and 5·99 ± 0·23, respectively. These data suggest that pharmacological responses of rat isolated lung strip to β-adrenoceptor agents are mediated by a homogeneous population of β_2-adrenoceptors, although the presence of a minor population of β_1-adrenoceptors undetected by the agonists used cannot be excluded.

We have previously studied the inhibitory effects of sympathomimetic amines on preparations of bathed as well as superfused lung strips of rat and the results obtained indicated that responses are mediated predominantly by β_2-adrenoceptors (Candenas et al 1986). β_2-adrenoceptors have also been found to be the only, or the predominant, β-adrenoceptor subtype involved in responses of lung strip preparations of other animal species, such as cat (Lulich et al 1976), pig (Goldie et al 1982) or guinea-pig (Carswell & Nahorski 1983). In rabbit lung strip, however, β_1-adrenoceptors seem to predominate (Chand & Deroth 1979).

To investigate further the β-adrenoceptor population within the rat lung strip, we have examined the antagonism by propranolol, atenolol and butoxamine of the effects of isoprenaline, salbutamol and noradrenaline, using the method described by O'Donnell & Wanstall (1979, 1981).

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Materials and Methods

Male Wistar rats, 200–260 g were killed by a blow on the head and exanguinated by sectioning of the carotid arteries. The lungs were removed and placed in modified Krebs-Henseleit solution maintained at 37°C. Strips of tissue approximately 10 mm in length and 2 mm in width, were cut from the centre of the lobe according to Lulich et al (1976). Strips were always dissected from the centre of the lobe since strips from the peripheral margin did not maintain a regular tone and did not show consistent pharmacological responses. The strips were mounted under a tension of 1 g in a 30 mL organ bath containing modified Krebs-Hanseleit solution that was maintained at 37°C and gassed continuously with a mixture of 95% O_2 and 5% CO_2. The composition of the physiological solution was (mM): NaCl 118·4; KCl 4·7; $CaCl_2$ 2·5; $NaHCO_3$ 25·0; KH_2PO_4 1·2; dextrose 11·1 and EDTA 0·04. The solution was adjusted to a pH between 7·2 and 7·4 with NaMOPS (morpholinopropane-sulphonic acid titrated with NaOH). The strips were allowed to rest for 1 h during which time the physiological solution was changed at 10 min intervals and tension was periodically readjusted to 1 g. Changes in tension was measured isometrically using a Gould Statham UC2 transducer and recorded on a HP 680M via an HP 8805C carrier amplifier.

Agonists were added to the bath in a cumulative manner, to obtain concentration-response curves. Only two curves to any given agonist were determined in any single experiment and in these conditions, preparations did not lose sensitivity. Hence, it was not necessary to introduce correction factors. An iterative computerized procedure (Basulto et al 1978) was applied to fit individual concentration-response curves and to calculate the maximum effect (E_{max}) and the concentration producing 50% of this maximum (EC50).

When antagonists were used, and after the dose-response curve to one agonist on three strips from the same animal had been established, a single and different concentration of an antagonist was added to each tissue, and after a 30 min