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Short Communication

Characterization of β-adrenergic receptors in the heart chambers of adult turkeys

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ABSTRACT

The presence, distribution and characteristics of chamber-specific β-adrenergic receptors in adult turkey hearts were investigated by radioligand binding studies using (−)-[^{125}I]-iodocyanopindolol (ICYP). The β_1-selective (CGP 20712A) and β_2-selective (ICI 118.551) antagonists as well as the nonselective β-agonists isoproterenol, epinephrine and norepinephrine were used in displacement studies.

In all cardiac chambers, ICI 118.551 and CGP 20712A displacement curves were monophasic and steep, with the affinity of CGP 20712A higher than that of ICI 118.551, indicating the exclusive presence of the β_1-adrenergic receptor subtype. The agonist rank order of potency was isoproterenol > norepinephrine ≥ epinephrine, typical for the β_1-receptor subtype. In all chambers, the density of β-adrenergic receptors was ~40 fmol/mg protein and the K_D was ~30 pM. The study revealed similar β-adrenergic receptor density mainly of the β_1-subtype in all cardiac chambers, indicating that this receptor subtype could contribute equally to regulate cardiac physiological function and pathophysiology.

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Co-existence of β_1- and β_2-adrenergic receptor subtypes, with a predominance and importance of the β_1-subtype, has been established in cardiac tissues of many species including humans (Brodde, 1991), cats and guinea pigs (Hedberg et al., 1980) and in rat atria (Kitagawa et al., 1995). Altered function and expression of the cardiac β_1-adrenergic receptor pathway have been reported in heart failure in humans (Brodde, 1991), whereas the role of the β_2-adrenergic receptor subtype is still being debated. Despite the fact that turkeys have been frequently used as an animal model to investigate human dilated cardiomyopathy (DCM) (Genao et al., 1996), β-adrenergic receptors have not been well characterised in the different cardiac chambers of turkeys. Moreover, in turkey farms there is considerable economic loss due to premature animal death (presumably caused by multifactorial diseases related to the cardiovascular system). The aim of this investigation was to characterise the β-adrenergic receptor density and subtype distribution in adult turkey heart chambers (right and left ventricles, right and left atria).

Heart samples were collected from 16-week old British United Turkey (BUT) BIG 6 female turkey poults which had been slaughtered at a local abattoir. Hearts were quickly transported to the laboratory and immediately dissected into four chambers. Samples were frozen at –80 °C prior to use. For the radioligand binding studies, membranes were prepared from all cardiac chambers (400 mg) as previously described (Seyfarth et al., 2000). The β-adrenergic receptor density and the relative amount of β_1- and β_2-adrenergic receptor subtypes were determined in cardiac membrane preparations by saturation and displacement binding studies using (−)-[^{125}I]-iodocyanopindolol (ICYP) and in the presence or absence of increasing concentrations (10^{-10}–10^{-4} M) of the β_1-receptor selective (CGP 20712A) or β_2-receptor selective (ICI 118.551) antagonists as well as nonselective β-agonists (isoproterenol, epinephrine and norepinephrine) as previously described (Abraham et al., 2003). All binding data were analysed using the iterative, nonlinear curve fitting GraphPad Prism software (GraphPad Software). Comparisons were made between chambers using analysis of variance (ANOVA) with post-hoc Bonferroni test, and $P < 0.05$ indicated the level of significance.

Specific ICYP binding was saturable and of high affinity (Figs. 1A–D). There was no statistical difference between β-adrenergic receptor density (B_{max}, ~40 fmol/mg protein) and dissociation constant (K_D; ~30 pM) in all cardiac chambers of adult turkeys. In all chambers, ICYP was displaced by ICI 118.551 and CGP 20712A in a concentration-dependent steep monophasic manner. The K_I values for CGP 20712A and ICI 118.551 are shown in Table 1. The agonist displacement curves for the right atria and ventricles were biphasic (Figs. 2A, B), with the rank order of potency as follows: isoproterenol > norepinephrine ≥ epinephrine.

Our data strongly suggest the exclusive presence of the β_1-adrenergic receptor subtype in adult turkey heart chambers. The fact that the concentration–inhibition curves of CGP 20712A and ICI 118.551 were steeply monophasic implies that both antagonists

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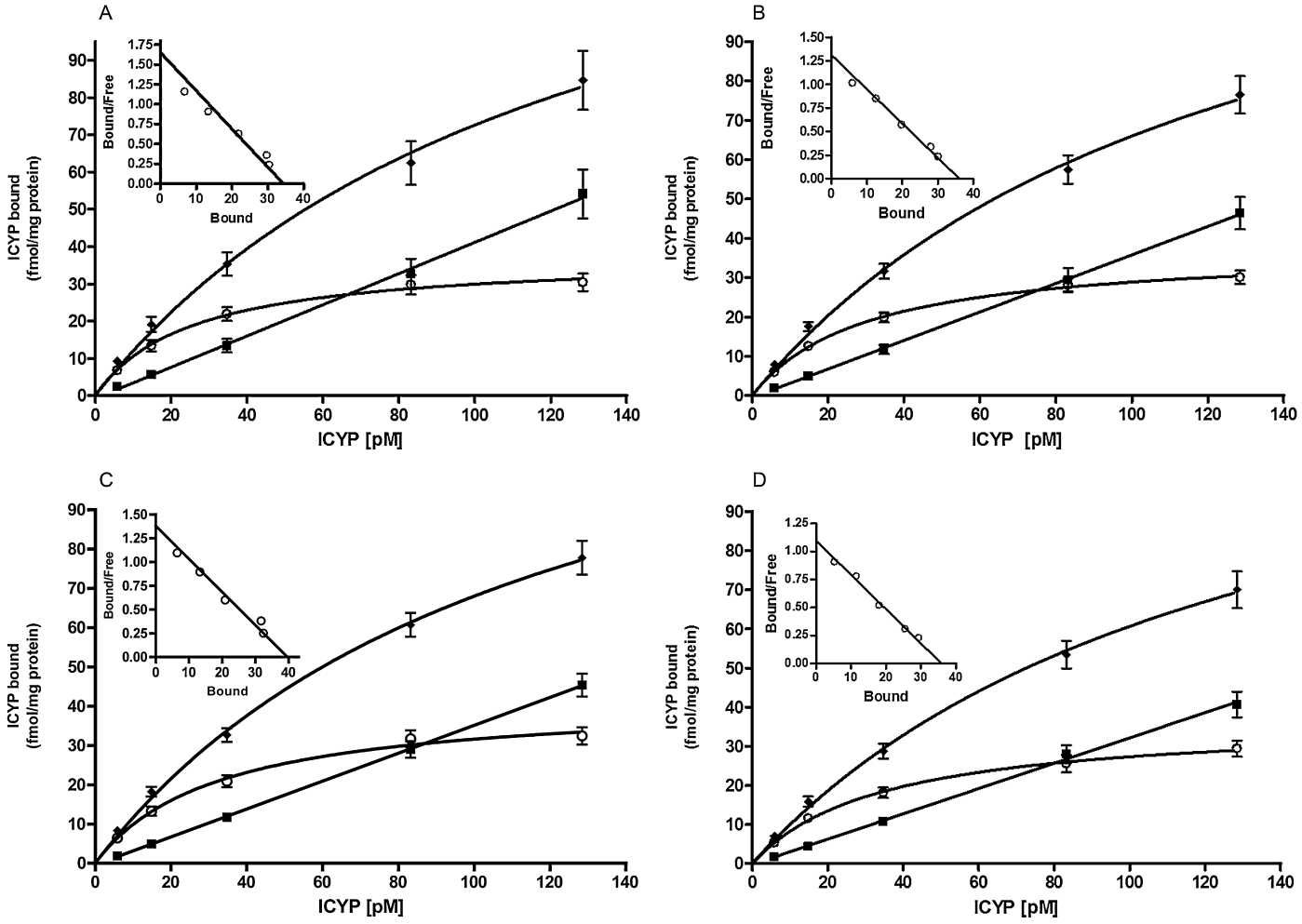

Fig. 1. Binding of iodocyanopindolol (ICYP) to crude membranes from the right atrium (A), left atrium (B), right ventricle (C) and left ventricle (D). Specific ICYP binding (○ SP) was calculated as the difference between total binding (♦ TB) and non-specific binding (· NSB). Each graph is representative of 20 experiments with data presented as means ± standard errors (SEM). Insets show the Scatchard plots of specific ICYP bindings, indicating a single binding site in each tissue.

displaced ICYP from one binding site. The selective β_1-adrenergic receptor antagonist CGP 20712A was highly potent in discriminating the β_1-receptor subtype, in accordance with the drug's selectivity for the subtype found in other pharmacological studies (K_I range, 10–100 nM; Dooley et al., 1986), but it was less potent in discriminating the β_2-receptor subtype (K_I range, >4000–10,000; Abraham et al., 2003). In contrast, ICI 118.551 was almost 25-fold less potent in discriminating the β_2-adrenergic receptors in turkey chambers when compared to other reported data (K_I range, <5 nM; Bilski et al., 1983; Abraham et al., 2003).

Table 1
Characteristics of antagonist binding to β-adrenergic receptors in membrane preparations of cardiac chambers of turkey poults.

Heart chambers	K_I ICI 118.551 (nM)	K_I CGP 20712 A (nM)
Right atrium	99.91 ± 17.68	103.33 ± 25.06
Left atrium	140.24 ± 32.37	112.22 ± 15.19
Right ventricle	109.93 ± 11.73	172.78 ± 21.62
Left ventricle	141.26 ± 22.82	172.82 ± 29.48

IC_{50} values were calculated from inhibition curves for each experiment, transformed into K_I values and given as the means ± standard errors (SEM) of eight experiments. All experiments were performed in duplicates. ANOVA analysis between chambers showed no statistical significance.

The rank order of agonist potency in turkey cardiac chambers demonstrated the predominant presence of the β_1-adrenergic receptor subtype, in line with the receptor classification by Lands et al. (1967). However, a previous radioligand binding study in the left ventricles of 7-week old turkeys reported the existence of both β_1- and β_2-subtypes at a ratio of 3:1 (Gwathmey et al., 1999). There are several explanations for this discrepancy. In contrast to our study, binding studies by Gwathmey et al. (1999) were carried out at 30 °C in the presence of the lipophilic β-adrenergic receptor antagonist propranolol. Furthermore, to determine β-adrenergic receptors, higher ICYP concentrations and less purified membrane preparations were used possibly impeding the exclusion of large amounts of nonspecific ICYP binding.

The β-adrenergic receptor density in the cardiac chambers of 16-week old turkeys was two times lower than the B_{max} obtained in the left ventricle of 7-week old turkeys, presumably for the reasons described above and the different ages of the birds (Gwathmey et al., 1999). To the best of our knowledge, there are no peer-reviewed studies available that describe the distribution and properties of β-adrenergic receptors in the four cardiac chambers of other avian species. The observation that the β_1-adrenergic receptor subtype is predominantly present in all cardiac chambers of turkeys suggests that heart dysfunction and treatments may be related to this receptor subtype, although this remains to be elucidated.

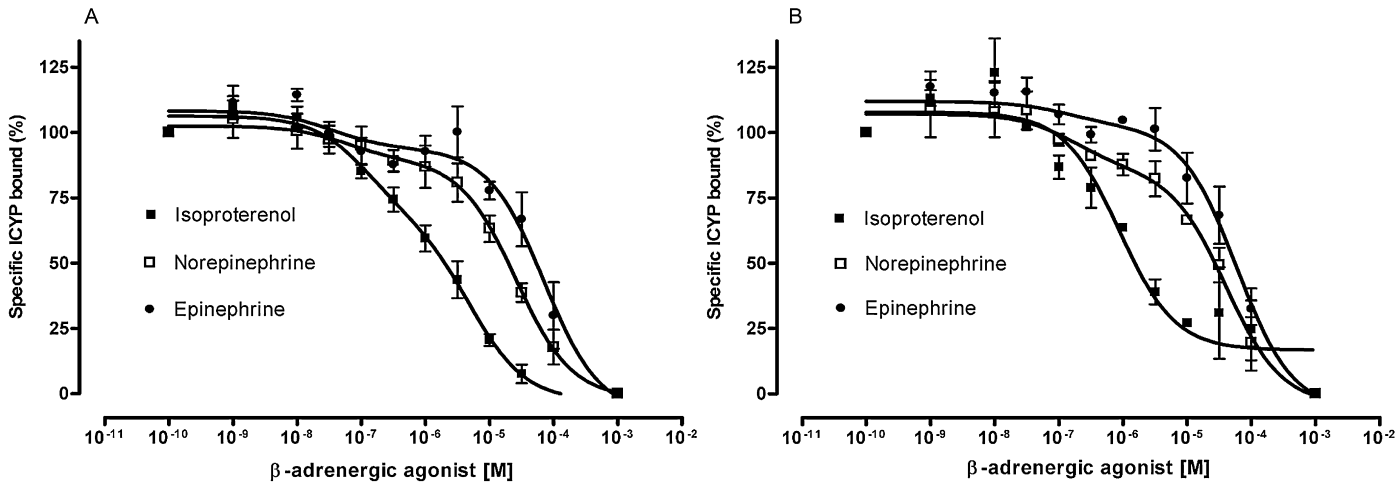

Fig. 2. Representative competitive displacement of iodocyanopindolol (ICYP) binding to crude membranes of the right ventricle (A) and right atrium (B) by isoproterenol, norepinephrine and epinephrine. Here, 100% binding represents the specific ICYP binding detected in the presence of 1 μM (±) CGP 12177 without the indicated agonists. Data shown represent the means ± standard errors (SEM) of three experiments, each performed in duplicates.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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