The effects of temperature and ionic environment on antagonist binding to cardiac β-adrenoceptors

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Previous studies (Pike & Lefkowitz, 1978) have suggested that temperature related changes in affinity for (−)[^{3}H]-dihydroalprenolol ([^{3}H]-DHA) binding to preparations of β-adrenoceptors are agonist specific; partial agonists and antagonists show no change in affinity with decreasing temperature. In this paper we describe an investigation of changes in temperature and ionic environment, on antagonist binding to cardiac β-adrenoceptors.

Pig hearts were obtained immediately after death and a microsomal fraction was prepared by a modification of the method of Bloomfield, Wells, Wellman & Peters (1977). Ten grammes of tissue were homogenised in 30 ml ice cold buffer (0.25 M sucrose, 10 mM $MgCl_2$, 50 mM Tris-HCl, 1 mM EDTA, pH 7.4 at 4°C), and after differential centrifugation, the microsomal pellet was resuspended in the incubation medium (50 mM Tris-HCl, 10 mM $MgCl_2$) at pH 7.4, with a final protein concentration of 500–800 μg/ml (Bradford, 1976).

[^{3}H]-DHA (48.6 Ci/mmole) was added (0.1–10 nM) in a final volume of 1 ml, following equilibrium, incubations were terminated by adding 2 ml ice-cold buffer followed by vacuum filtration through Whatman GF/B glass fibre filters, and 3 × 4 ml washes with ice-cold buffer. 'Specific binding' of [^{3}H]-DHA was defined as that displaceable by (−)-propranolol (10^{-5} M and represented 80–90% of total radioactivity-bound. Binding parameters were determined by least squares analysis of Scatchard plots (1949).

In order to investigate the ionic component of [^{3}H]-DHA binding, varying concentrations of NaCl (0, 30, 150 and 300 mM) were included in the incubation buffer, and equilibrium binding characteristics determined at 0° and 37°C. At 0°C, increasing NaCl concentrations from 0 to 300 mM produced a marked increase in binding capacity (B_{max} increase = 106 fm mg protein^{-1}, range 77–135, $n = 2$) which may have been due to ionic changes in the membrane unmasking new sites for [^{3}H]-DHA, as has been reported for insulin receptors (Cuatrecasas, 1971). A small increase in K_D was also observed (K_D increase = 0.84 nM range 0.06–1.61 nM, $n = 2$). At 37°C, increasing the NaCl concentration from 0 to 300 mM had less effect on binding capacity but produced a fall in K_D (B_{max} increase = 64.66 fm mg protein^{-1} ± 75.9 s.e. mean, K_D decrease = 2.84 nM ± 0.31 s.e. mean, $n = 3$). This increase in affinity with increasing ionic environment would suggest the involvement of hydrophobic forces.

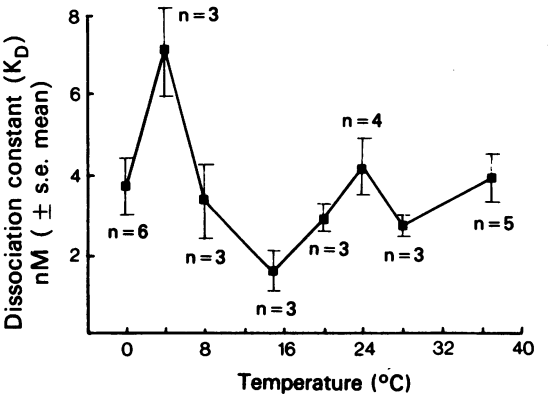

Figure 1.

K_D also varied with temperature (Figure 1) and this may be due to changes in membrane fluidity, as have been reported for cholesterol/phospholipid/water mixtures (Bangham, 1972; Engelman & Rothman, 1972) in the temperature range 15–20°C, whereas the marked fall in binding affinity at 4°C could be associated with changes in water structure (Belleau, 1966).

Thus these findings would indicate that under physiological conditions hydrophobic forces may be important. This is further supported by thermodynamic analyses of the temperature dependency of K_D, a large change in heat capacity (−0.453 Kcal deg^{-1} mole^{-1}) and accompanying decreases in enthalpy and entropy occurring with increasing temperature, being characteristic of hydrophobic bond formation.

(−)-Propranolol was a gift from ICI Ltd. D.S.M. is an M.R.C. student.

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Cocaine and desipramine antagonize the clonidine-induced inhibition of [^{3}H]-noradrenaline release from the rat cerebral cortex

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Imidazolines like clonidine are known to reduce the stimulation-evoked [^{3}H]-noradrenaline ([^{3}H]-NA) overflow from noradrenergic nerve endings through activation of presynaptic alpha-adrenoceptors, which mediate a negative feed-back mechanism in the peripheral as well as in the central nervous system (CNS) (Langer, 1977; Starke, 1977).

In the perfused cat spleen the inhibition of the stimulation-evoked release of [^{3}H]-NA by the imidazoline oxymetazoline is antagonized by two inhibitors of neuronal uptake: amphetamine and cocaine (Dubocovich, Langer & Moret, 1979). Consequently it was considered of interest to investigate a similar type of interaction in the CNS.

Male rats were killed by decapitation and slices prepared from the cerebral cortex were labelled *in vitro* by incubation with (±)-[^{3}H]-NA (0.5 μM). Slices were then superfused with Krebs solution and the release of [^{3}H]-NA was elicited by a 2 min period of electrical stimulation at a frequency of 3 Hz. In the controls the fraction of total tissue tritium released (FR) by the first period of stimulation (S_1) was: 3.41 ± 0.21 ($\times 10^2$) ($n = 23$). The ratio of FR between two consecutive stimulation periods S_2/S_1 was: 0.90 ± 0.05 ($n = 6$). Under these experimental conditions the release of [^{3}H]-NA was found to be entirely calcium-dependent. Clonidine reduced the stimulation-evoked [^{3}H]-NA release in a concentration-dependent manner. The inhibition of [^{3}H]-transmitter release was: 33.8 + 7.5% ($n = 7$); 57.1 ± 5.3% ($n = 5$) and 65.5 ± 4.4% ($n = 5$) for 0.03, 0.1 and 1 μM of clonidine respectively.

When cocaine (10 μM) was present in the superfusion medium throughout the experiment, the value of FR in S_1 was increased to: 7.44 ± 0.56 ($\times 10^2$) ($n = 12$, $P < 0.001$ when compared with the control values) and the ratio between two consecutive stimulation periods S_2/S_1 was: 1.06 ± 0.07 ($n = 3$). In the presence of cocaine (10 μM) the inhibition of [^{3}H]-NA release obtained with clonidine (0.1 and 1 μM) was only 24.2 + 7.9% ($n = 6$) and 14.8 ± 10.7% ($n = 4$) respectively. These effects were significantly smaller ($P < 0.01$) than those obtained in the absence of cocaine.

When desipramine (DMI, 0.1 μM) was present in the superfusion medium throughout the experiment the value of FR in S_1 was 5.18 ± 0.27 ($\times 10^2$) ($n = 24$, $P < 0.001$ when compared with the control values) and the ratio S_2/S_1 was: 1.21 ± 0.09 ($n = 5$). In the presence of DMI (0.1 μM) the inhibition of [^{3}H]-NA release obtained with clonidine (0.1 and 1 μM) was: 27.1 ± 5.8% ($n = 5$) and 38.2 ± 7.1% ($n = 4$) respectively. These reductions were significantly smaller ($P < 0.01$) than those observed in the absence of DMI. It is of interest to note that in the controls the alpha-adrenoceptor antagonist phentolamine (1 μM), significantly increased the stimulation-evoked [^{3}H]-NA release (S_2/S_1: 3.85 ± 0.77, $n = 6$, $P < 0.01$ when compared with controls). In the presence of DMI (0.1 μM) throughout the experiment phentolamine (1 μM), added before S_2 produced an increase of similar magnitude to that obtained in the absence of DMI (S_2/S_1: 3.61 ± 0.53, $n = 4$).

When neuronal uptake of (±)-[^{3}H]-NA (0.05 μM) was studied in rat cerebral cortex slices it was found to be inhibited by 80.5%, $n = 4$ and 81.0%, $n = 4$ in the presence of DMI (0.1 μM) and cocaine (10 μM) respectively. The increase in the stimulation-induced [^{3}H]-NA overflow obtained in the presence of cocaine and DMI is most probably due to the inhibition of NA uptake. Under our experimental conditions the antagonism by DMI of the clonidine induced inhibition of neurotransmission cannot be attributed to a blockade of presynaptic alpha-adrenoceptors by DMI because the facilitating effect on [^{3}H]-NA release by phentolamine was not modified in the presence of this drug.

It is concluded that an increase in the concentration of NA in the synaptic gap as obtained when neuronal uptake is inhibited may explain the decreased effectiveness of clonidine in reducing nor-