The subcellular binding of propranolol in rat heart

SIR,—Propranolol is known to block β-receptor actions upon the heart (Black, Duncan & Shanks, 1965; Nakano & Kusakari, 1965). We now report the binding of [^{3}H]propranolol to various particles in the heart of the rat.

^{3}H-Labelled propranolol (250 and 500 μc; specific activity 0·94 mc/mg) was injected into the tail vein of male Sprague-Dawley rats. After 30 min, each rat was decapitated and the heart quickly removed, rinsed and chilled, and homogenized in 5 volumes of ice-cold isotonic sucrose solution (0·25 M). Subcellular fractionation in a continuous sucrose gradient and subsequent determination of radioactivity was made (Potter & Axelrod, 1963a,b). To prepare the gradient, 4·5 ml of 0·25 M sucrose was placed in the upper tube of an exponential gradient maker and 4·5 ml of 2·2 M sucrose into the lower flask. The tube containing the gradient was chilled, 0·5 ml of the heart homogenate was layered over the gradient and this was then centrifuged in a Spinco model L preparative ultracentrifuge at an average force of 125,000 *g* for 30 min. All homogenization and fractionation procedures were at 0°– 4°. Although the fractions were not verified by electron microscopy, the layers in the gradient tube after centrifugation corresponded visually to that described and illustrated by Potter & Axelrod (1963a).

Fig. 1A illustrates the distribution of tritium label in the density gradient tube after centrifugation of a homogenate of a heart removed from a rat 30 min after intravenous injection of [^{3}H]propranolol. Radioactivity was found to be predominantly associated with the "microsomal" fraction and to a lesser extent with the mitochondrial and muscle debris layers. However, when heart tissue from a non-injected rat was homogenized in 0·25 M sucrose with [^{3}H]-propranolol (0·5 μc), a distribution pattern similar to that illustrated in Fig. 1A resulted.

That this binding property of propranolol is not shared by (±)-[^{14}C]noradrenaline* (^{14}C-NA) and is not solely a result of our technique is demonstrated in Fig. 1B. It is seen that noradrenaline possesses markedly different binding

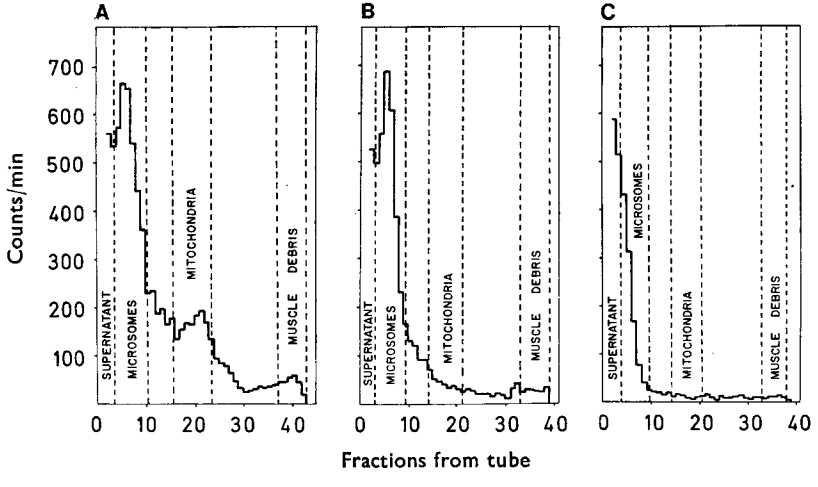

FIG. 1. Subcellular distribution of radioactivity in rat heart 30 min after intravenous injection of (A) [^{3}H]propranolol, (B) [^{14}C]noradrenaline, (C) density gradient fractionation of rat heart homogenized with [^{14}C]noradrenaline.

characteristics. Thirty min after intravenous injection, ^{14}C-NA (2 μc; specific activity 43 mc/mM) is localized almost exclusively in the "microsomal" fraction of rat heart, confirming the findings of Potter & Axelrod (1963a). Also, in contrast to propranolol, ^{14}C-NA (6×10^{-4} μc) shows no peak of radioactivity associated with any particulate fraction when mixed and homogenized with non-labelled heart tissue in sucrose (Fig. 1C).

To exclude the possibility that the larger quantity of propranolol used might have been responsible for general labelling of all particulate fractions, (±)-[^{3}H]noradrenaline* (specific activity 10·28 c/mM) was diluted with sufficient non-radioactive noradrenaline to have the same specific activity as that of [^{3}H]propranolol, and the same quantity of radioactivity was mixed and homogenized in 0·25 M sucrose with heart tissue from a non-injected rat. This distribution was likewise similar to Fig. 1C. Because of toxicity the *in vivo* experiment with a noradrenaline dose comparable to that of injected propranolol could not be made.

These experiments demonstrate the markedly different binding property of propranolol and noradrenaline. The distribution picture appears to indicate that noradrenaline is specifically and actively taken up by particles of microsomal size, which confirms other reports (Potter & Axelrod, 1963b; Sjöqvist, Titus & others, 1965; Taylor, Chidsey & others, 1966), whereas propranolol, which is lipid soluble, appears to bind indiscriminately to various membrane structures. In the *in vivo* experiment, the possibility cannot be excluded that the subcellular localization pattern of propranolol was actually different *in vivo* and that a subsequent redistribution might have occurred during homogenization.

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* 2-Amino-1-(3,4-dihydroxyphenyl)-[1-Label]ethanol.