the possibility that propranolol itself may be taken up into synaptosomes by the same mechanism responsible for noradrenaline uptake.

Whole brains were removed from male Wistar rats (250–350 g) and crude synaptosomes (P_2 fraction) were prepared by the method of Whittaker, Michaelson & Kirkland (1964). After separation, the P_2 pellet was resuspended in ice-cold Krebs-Ringer phosphate buffer, and this suspension was used for the incubations. Incubations were performed at 37°C for 7 min, and the incubation medium contained 0.1 ml of the synaptosome suspension in a total volume of 2.0 ml Krebs, incorporating (−)-[^{3}H]-noradrenaline hydrochloride or (±)-[^{14}C]-propranolol hydrochloride. Glucose (10 mM), ascorbic acid (0.2 mg/ml), EDTA (0.1 mg/ml) and nialamide (1.25×10^{-5} M) were also present. Incubations were terminated by cooling and centrifugation, and the synaptosomes were then washed, solubilised and assayed for total radioactivity by liquid scintillation spectrometry.

[^{3}H]-noradrenaline was taken up into synaptosomes by a saturable high-affinity uptake process, with a K_m of 0.28 μM and a V_{max} of 5.7 pmoles NA mg protein^{-1} minute^{-1}. At a substrate concentration of 1.0×10^{-7} M, uptake was found to be Na^+-dependent and temperature-sensitive. At the same substrate concentration, cocaine (1.6×10^{-7} M), desipramine (1.7×10^{-7} M), ouabain (2.8×10^{-6} M), propranolol (1.9×10^{-5} M), oxprenolol (2.8×10^{-5} M) and metoprolol (8.8×10^{-5} M) were all inhibitors of uptake, and the IC_{50} values for these inhibitors are shown in parentheses. In contrast, the uptake of [^{14}C]-propranolol, at a substrate concentration of 8.5×10^{-7} M, was not affected by extracellular Na^+ concentration, or by cocaine, desipramine or ouabain at the previously mentioned IC_{50} concentrations. Propranolol uptake was reduced by approximately 30% at 4°C, but this may possibly be explained by the temperature-dependent partitioning of this drug (Street, 1979).

In these experiments, propranolol was an effective inhibitor of synaptosomal noradrenaline uptake, but did not itself appear to be taken up into synaptosomes by the same high-affinity process responsible for the uptake of noradrenaline. It is suggested that the incorporation of propranolol into synaptosomal fractions of rat brain homogenates is largely a function of its lipophilicity.

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The neuromuscular blocking action of some cyclic analogues of choline

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In this study four analogues of hemicholinium-3 (HC-3) have been synthesised, where the interatomic distance between the two morpholinium rings is increased by the insertion of methylene groups, $(CH_2)_n$ where $n = 1$ to 4, between the two phenyl rings. The compound where $n = 0$ is HC-3 itself.

The neuromuscular blocking action of these compounds has been investigated using the rat phrenic nerve-hemidiaphragm preparation (Bulbring, 1946). All the analogues gave a prejunctional block which was reversed by choline (0.1 μm/ml).

A partially purified extract of choline acetyltransferase (ChAC) was obtained from rat brain and incubated at 37°C with [^{14}C]-acetyl CoA and either choline or one of the analogues (20 mM). The amount of acetylation was determined in each case.

A similar incubation system was used to measure

the inhibitory action of the analogues on ChAc. When no inhibitor was present the inhibition was 0%.

Synaptosomes (P_2 fraction of Gray & Whittaker, 1962) were prepared from rat brain and incubated with [^{3}H]-choline. All the analogues inhibited the high affinity transport of choline into synaptosomes.

The analogues where $n = 2$, 3 and 4 are more potent inhibitors of choline transport into synaptosomes when compared to the other two compounds. This probably contributes to their more potent presynaptic block, in the rat phrenic nerve-diaphragm preparation.

The analogue where $n = 3$ is acetylated in vitro by ChAc at a similar rate as HC-3 itself. Using Michaelis-Menten kinetics and apparent K_m values were calculated: HC-3, 1.21 mM; $n = 3$, 1.27 mM. The values for V_{max} (μm of ^{14}C-acetylated product g of enzyme 10 mins) were: HC-3, 4.2; $n = 3$, 7.95. (Conc. of acetyl CoA, 2.3×10^{-5} M).

Because both HC-3 and the analogue where $n = 3$ are acetylated by ChAc in vitro, it is possible that the acetylated products so formed could be released as false cholinergic transmitters *in vivo*.

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