The stereospecificity of noradrenaline uptake by cat atria

SIR,—Kopin & Bridgers (1963) noted that there was a difference in the rate of disappearance of (±)-[^{3}H]noradrenaline from rat hearts *in vivo* when compared with (−)-[^{14}C]noradrenaline, and this was shown subsequently to be due to different binding forces or rates of release for the (+)- and (−)-isomers of noradrenaline (Beaven & Maickel, 1963). Iversen (1963, 1965) showed that the uptake kinetics for (+)- or (−)-noradrenaline into whole rat hearts were also different at low concentrations of noradrenaline (less than 0·5 μg/ml), but were not dissimilar at higher concentrations. Since we previously reported a lack of stereospecificity for (+)- or (−)-noradrenaline uptake into particulate fractions of cat atria using high total noradrenaline concentrations (Mueller & Shideman, 1964), it was felt desirable to see if in this species stereospecificity could be observed at low total noradrenaline concentrations. Because specificity could exist at either the cell membrane or the storage vesicle membrane, or at both, it was desirable to fractionate the atrial homogenates obtained after exposure of the intact spontaneously beating organ to (±)-7[^{3}H]noradrenaline* [^{3}H]-NA. Since adequate control of exposure to and washout of [^{3}H]-NA from tissues seemed essential, isolated superfused cat atria were employed. In the superfused preparations the atria were bathed only by solution flowing over the outside of the tissue.

Cats of either sex (1–2 kg) were anaesthetized with sodium pentobarbitone (35 mg/kg i.p.). The heart was removed rapidly and placed in an oxygenated modified Tyrode solution having the following composition in g/litre: NaCl, 7·00; KCl, 0·354; $CaCl_2.2H_2O$, 0·350; KH_2PO_4, 0·081; $MgSO_4.7H_2O$, 0·147; $NaHCO_3$, 2·1; glucose, 0·9; ascorbic acid, 0·02. The atria were dissected free from ventricular muscle and placed in a superfusion chamber similar to that described by Gaddum (1953) and Cambridge & Holgate (1955). The muscle was covered with 100 ml of the modified Tyrode solution, and a resting load of 1·0 g was applied. After superfusion was begun, the bath was drained and the resting tension was increased by the weight of the atria under study. The rate of superfusion was held constant with two Dual Syringe Feeders (Modern Metalcraft) which delivered 3·5 to 3·8 ml/min. Atria were first superfused for 10 or 50 min with modified Tyrode solution, then exposed to modified Tyrode solution containing the [^{3}H]-NA, and finally washed for 3 min with solution containing no [^{3}H]-NA. The atria were rapidly removed from the apparatus, blotted dry, and homogenized in 0·075 M phosphate buffer (pH 7·5). An initial sediment was prepared by centrifuging the homogenates at 5000 × *g* for 5 min (IS); the supernatant fluid was then centrifuged at 105,000 × *g* for 60 min yielding a pellet (P) and final supernatant (S) fraction. Since the [^{3}H]-NA isolation procedure (Whitby, Axelrod & Weil-Malherbe, 1961) does not separate (+)- from (−)-[^{3}H]-NA, the estimated radioactivity reflects the amounts of both labelled isomers taken up by the tissue fractions. The (−)-isomer of noradrenaline was obtained from Calbiochem Corporation as the (−)-noradrenaline (+)-bitartrate monohydrate $[\alpha]_D^{25}$ −9·6, and the (+)-isomer of noradrenaline was obtained from Sterling Winthrop Laboratories as the (+)-noradrenaline (+)-bitartrate $[\alpha]_D^{25}$ +36·8.

At 1 μg/ml of (+)- or (−)-noradrenaline, 2·3 ng/ml of (±)-[^{3}H]-NA (1 μc/86 ng) was added to the superfusing medium, resulting in an 881-fold dilution of the isomer under study. At concentrations of 20 ng/ml of (+) or (−)-noradrenaline, 2·9 ng/ml of (±)-[^{3}H]-NA (1 μc/29 ng) was added to the superfusing fluid resulting in a 14-fold dilution of the (+)- or (−)-isomer.

* 2-Amino-1-(3,4-dihydroxyphenyl)-[1-^{3}H]ethanol.

When atria were superfused for 10 min with modified Tyrode solution containing (±)-[^{3}H]-NA after a 50 min control superfusion period (Table 1, Exp. series 1), dilution of either (−)- or (+)-[^{3}H]-NA contained in the superfused fluid by addition of large amounts (1 μg/ml) of unlabelled (+)- or (−)-noradrenaline caused an equal depression of the [^{3}H]-NA taken up into the P fraction. Since it was thought possible that a 50 min period of control superfusion might destroy a stereospecific mechanism, the experiment was repeated using only a 10 min period of superfusion before superfusing with Tyrode solution containing [^{3}H]-NA (Experimental series 2). Under these conditions the amount of [^{3}H]-NA taken up was significantly lower than with 50 min control period of superfusion. However, the addition of 1 μg/ml of unlabelled (+)- or (−)-noradrenaline again produced an equal depression of the amount of (+)-[^{3}H]-NA taken up into the P fraction and the total [^{3}H]-NA taken up by the heart. The experiment was repeated again (Experimental series 3) using the 10 min control period and a much smaller final concentration of diluting unlabelled (+)- or

TABLE 1. THE EFFECT OF (+)- AND (−)-NORADRENALINE ON UPTAKE AND SUBCELLULAR DISTRIBUTION OF (±)-[^{3}H]NORADRENALINE IN SUPERFUSED CAT ATRIA. Cat atria were superfused with modified Tyrode solution during the control period. All atria were superfused with the solution containing [^{3}H]-NA for 10 min. Atrial homogenates were fractionated by an initial sedimentation at 5000 × *g* for 5 min (IS); the supernatant thus obtained was sedimented at 105,000 × *g* for 60 min, yielding a pellet (P), and final supernatant solution (S).

Exp. ser.	No. of exp.	Control period before [^{3}H]-NA (min)	Unlabelled isomer of NA added to superfusing solution	Conc. isomer μg/ml	Total [^{3}H]-NA mμc/g in atria at end of superfusion mean ± s.e.	% of total [^{3}H]-NA (Mean ± s.e.) Subcellular fraction IS	P	S
1	4	50	None		68·4 ± 8·6	15·8 ± 0·5	12·2 ± 0·6	72·3 ± 0·8
	4	50	(−)	1	63·3 ± 10·5	20·3 ± 2·1	6·0 ± 1·1[a]	73·8 ± 1·8
	4	50	(+)	1	63·3 ± 11·2	20·0 ± 3·0	7·0 ± 0·8[a]	73·0 ± 2·5
2	5	10	None		26·9 ± 0·7	17·2 ± 0·8	18·1 ± 1·4	64·7 ± 0·6
	7	10	(−)	1	15·8 ± 2·5[a]	22·8 ± 5·5	13·4 ± 1·3[a]	63·9 ± 5·3
	5	10	(+)	1	14·5 ± 1·0[a]	17·9 ± 2·0	11·8 ± 4·6[a]	70·3 ± 6·5
3	4	10	None		21·4 ± 1·2	11·4 ± 0·3	21·6 ± 1·1	67·0 ± 1·1
	6	10	(−)	0·02	11·3 ± 0·8[a]	14·3 ± 2·0	14·8 ± 0·9[a]	71·0 ± 2·3
	4	10	(+)	0·02	21·2 ± 2·3	11·4 ± 1·0	24·1 ± 1·2	64·7 ± 1·3

[a] Indicates value significantly different ($P < 0·05$) from comparable value of hearts superfused with (±)-[^{3}H]-NA containing neither added (+)- or (−)-noradrenaline.

(−)-isomer. In this series the (−)-isomer caused a highly significant depression of both the percentage uptake into the P fraction, and in the total [^{3}H]-NA taken up per gram of tissue. Neither change was seen when unlabelled (+)-isomer was used to dilute the labelled (+)-isomer of (±)-[^{3}H]-NA. This would indicate sterospecific uptake both at the cell membrane and the storage vesicle membrane.

These results indicate that the myocardial adrenergic nerve terminals of the cat, like those of the rat (Iversen 1963, 1965) and isolated bovine splenic nerve granules (Euler & Lishajko, 1964; Stjärne & Euler, 1965) exhibit stereospecificity only at low concentrations of noradrenaline, but not at high concentrations. This could be the result of 2 separate uptake mechanisms or one mechanism whose characteristics are such that at high concentrations of noradrenaline stereospecificity is not detectable.

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The effect of (±)-*p*-chloroamphetamine on the susceptibility to seizures and on the monoamine level in brain and heart of mice and rats

SIR,—In recent experiments we established that α-methyldopa inhibits the convulsion-facilitating effect and the brain noradrenaline-depleting effect of reserpine. The 5-hydroxytryptamine (5-HT)-depleting effect of reserpine was not influenced by α-methyldopa (Pfeifer & Galambos, 1965). We assumed that the changes in brain noradrenaline level played an important role in the susceptibility to seizures but that changes in the 5-HT level did not. Pletscher, Bartholini & others (1964) and Fuller, Hines & Mills (1965) reported the fact that *p*-chloro-*N*-methylamphetamine and *p*-chloroamphetamine lowered the 5-HT level in rat brain without lowering the concentration of noradrenaline. The compounds did not decrease the 5-HT level in the brain in mice. On the basis of these observations *p*-chloroamphetamine seemed to be a useful tool for investigating further the role of 5-HT and catecholamines in the susceptibility to seizures.

Wistar rats and Swiss mice were used in these experiments. The convulsive threshold was determined by the slow intravenous infusion of leptazol (Orloff, Williams & Pfeiffer, 1949). Noradrenaline, dopamine and 5-HT levels in brain and heart were measured by spectrophotofluorimetry (Bogdanski, Pletscher & others, 1956; Drujan, Sourkes & others, 1959).

(±)-*p*-Chloroamphetamine (10 mg/kg) much increased the convulsive threshold in mice and also in rats after 30 min, and the effect was seen even after 8 hr. There was no change in the brain 5-HT levels in mice. In rats the brain 5-HT level decreased to about 40%, and even after 18 hr when the convulsive threshold had returned to the control value, the 5-HT level was still low (Table 1). The brain noradrenaline and dopamine levels in rats and mice were unchanged.

The anticonvulsive effect of (±)-*p*-chloroamphetamine also developed in the presence of reserpine. The mice received 2·5 mg/kg reserpine intraperitoneally and 90 min later chloroamphetamine 10 mg/kg. The leptazol convulsion threshold was estimated after 30 min. In these circumstances the convulsion-facilitating effect of reserpine was not seen. When the mice were treated with chloroamphetamine 2 hr before reserpine and the convulsive threshold was