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STUDIES ON THE UPTAKE AND RELEASE OF PROPRANOLOL AND THE EFFECTS OF PROPRANOLOL ON CATECHOLAMINES IN CULTURES OF BOVINE ADRENAL CHROMAFFIN CELLS*

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Abstract—Uptake and release of [^{3}H]*l*-propranolol and the effects of propranolol on the uptake and release of [^{3}H]norepinephrine were studied in cultures of isolated bovine adrenal chromaffin cells. [^{3}H]*l*-Propranolol uptake increased with increasing [^{3}H]*l*-propranolol concentration from 10^{-7} M to 10^{-3} M and was not saturable in this concentration range. [^{3}H]*l*-Propranolol uptake was equally inhibited by *l*- and *d*-propranolol, indicating that the uptake is not stereoselective. [^{3}H]*l*-Propranolol uptake differed from [^{3}H]norepinephrine uptake in two respects: (1) [^{3}H]*l*-propranolol uptake was 44–50 times greater than [^{3}H]norepinephrine uptake at early non-equilibrium time periods, and (2) [^{3}H]*l*-propranolol uptake was not Na^+ dependent and was not inhibited by desipramine, indicating that [^{3}H]*l*-propranolol is not taken up by the biogenic amine transport system. In cells preloaded with [^{3}H]*l*-propranolol, two agents, veratridine and tyramine, stimulated an increased release of [^{3}H]*l*-propranolol into the medium. However, veratridine-induced [^{3}H]*l*-propranolol release was inhibited only slightly by the Na^+ channel blocker tetrodotoxin, and tyramine-induced [^{3}H]*l*-propranolol release was not inhibited by desipramine. In addition, K^+, carbachol and the physiological mediator of adrenal catecholamine release, acetylcholine, failed to evoke [^{3}H]*l*-propranolol release. Therefore, it is unlikely that propranolol is released in response to physiological stimulation of adrenal chromaffin cells in animals administered propranolol *in vivo*. *l*-Propranolol inhibited [^{3}H]norepinephrine uptake by chromaffin cells with an IC_{50} for *l*-propranolol of 5×10^{-6} M; *d*-propranolol was equally potent for this effect at lower propranolol concentrations. By themselves, neither *l*- nor *d*-propranolol had any effect on [^{3}H]norepinephrine release from the cells. However, *l*-propranolol inhibited carbachol-induced [^{3}H]norepinephrine release with an IC_{50} for *l*-propranolol of 5×10^{-7} M to 10^{-6} M. At these lower concentrations, *d*-propranolol had no effect on carbachol-induced [^{3}H]norepinephrine release, indicating that the inhibition by *l*-propranolol may be mediated via β-adrenoceptors on chromaffin cells.

Recent evidence suggests that part of the metabolic disposition of propranolol may include uptake of propranolol by both peripheral adrenergic and central nervous tissue and release of the drug in response to stimulation of these tissues. Peripherally, in dogs chronically pretreated with propranolol, electrical stimulation of the cardioaccelerator nerves, the lumbar sympathetic chain or the splenic nerves caused an increased outflow of propranolol into the perfusate from the target organs of these nerves [1, 2]. In these preparations, the release of propranolol in response to stimulation appeared to be due, at least in part, to release of the drug presynaptically from adrenergic nerves. In support of this, sympathetic ganglia cultured in the absence of their target organs have also been shown to accumulate [^{3}H]propranolol and release the drug in response to stimulation with tyramine or the depolarizing agent veratridine [1]. As regards central nervous tissue, [^{3}H]propranolol can be taken up by synaptosomes from rat cerebral cortex and is released by depolarization of the tissue with K^+ or veratridine [3]. These results suggest, then, that part of the pharmacological effects of propranolol may be due to release of the drug from adrenergic nerves and accumulation of high concentrations of the drug in the synaptic cleft during nerve activity [1, 3].

Experiments investigating the effects of propranolol on catecholamine release and uptake indicate that propranolol may affect adrenergic neurotransmission in at least three different ways. (1) Propranolol has been shown to inhibit norepinephrine (NE§) release during stimulation of several preparations including isolated atria [4], vas deferens [5–7], ileum [8], ear arteries [5–8] and nictitating membrane [9]. This inhibition of stimulation-induced NE release has been attributed to blockade of β-adrenoceptors normally mediating a positive feedback on NE release from sympathetic nerve endings at lower concentrations of propranolol, and to the local anaesthetic properties of propranolol, at higher drug concentrations. (2) Propranolol by itself is able to stimulate NE release from adrenergic nerve endings in the heart [10] and vas deferens [7]. This action of propranolol may be due to direct displacement of

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§ Abbreviations: ACh, acetylcholine; E, epinephrine; and NE, norepinephrine.

NE stores by propranolol within synaptic vesicles [3]. (3) Propranolol and other β-adrenoceptor blocking drugs have also been shown to inhibit NE uptake by the heart [11] and by cerebral cortex slices [12, 13] and synaptosomes from whole brain [14].

Another adrenergic tissue in which propranolol may be stored, released and interact with catecholamine metabolism is the adrenal medulla. This idea is supported by observations that propranolol treatment can alter plasma epinephrine (E) levels in man [15, 16]. Furthermore, Sugawara *et al.* [17] reported that, in anaesthetized rats, intravenous propranolol causes an increase in E, NE and dopamine in the adrenal venous blood and this increase is blocked by adrenalectomy. These investigators suggested, from indirect evidence, that the increase in venous catecholamines was due, in part, to a direct effect of propranolol to release catecholamines from the adrenal. Experiments with isolated adrenal chromaffin cells allow one to assess the direct effects of propranolol on these cells. In studies with this preparation, Greenberg and Zinder [18] showed that racemic *d,l*-propranolol inhibits the acetylcholine (ACh)-stimulated release of catecholamines from chromaffin cells, indicating that propranolol does have one direct effect on adrenal medullary cells. In the present study, I have investigated the uptake and release of [^{3}H]*l*-propranolol by cultures of isolated adrenal chromaffin cells. I have further examined the interactions of propranolol with adrenal catecholamine metabolism by studying the effects of *l*-propranolol on [^{3}H]NE uptake and on basal and carbachol-stimulated [^{3}H]NE release in these cultures. These effects of *l*-propranolol were also compared to those of *d*-propranolol, which is generally much less active than the *l*-isomer as a β-adrenoceptor antagonist.

MATERIALS AND METHODS

Materials. *l*-[4-^{3}H]Propranolol (sp. act. 16.6 Ci/mmole) and *l*-[7-^{3}H(N)]norepinephrine (sp. act. 24.8 Ci/mmole) were purchased from New England Nuclear, Canada, and acetylcholine and carbachol were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. *Laevo*-propranolol and *dextro*-propranolol were gifts from Imperial Chemical Industries PLC, Macclesfield, England.

Chromaffin cell cultures. Chromaffin cells were isolated by retrograde perfusion of adult bovine adrenal medullae with DNAse I and collagenase, and purified by density gradient centrifugation in Percoll, as described by Trifaró *et al.* [19] and Trifaró and Lee [20]. Purified chromaffin cells were plated on collagen-coated plastic Multiwell plates (Falcon) at a density of 2.5×10^5 cells/well. The cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, cytosine arabinoside (10^{-5} M) and antibiotics (penicillin, 200 μg/ml; streptomycin, 50 μg/ml; gentamycin sulfate, 10 μg/ml; mycostatin, 25 units/ml), and the medium was changed every 3–4 days. The cultures were incubated at 37° in a humid atmosphere gassed with 5% CO_2 in air for 5–14 days until use.

Except where noted, all of the following experiments were performed at 23°, and the composition of the standard Krebs–Ringer buffer (pH 7.4) was as follows: NaCl (125 mM), KCl (4.8 mM), *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (25 mM), $MgSO_4$ (1.2 mM), KH_2PO_4 (1.2 mM), glucose (5.6 mM), and $CaCl_2$ (2.2 mM).

[^{3}*H*]*l-Propranolol and* [^{3}*H*]*NE uptake.* Each culture was washed three times with 0.5 ml buffer and pre-equilibrated for at least 5 min in 0.5 ml buffer. The culture was then incubated with [^{3}H]*l*-propranolol or [^{3}H]NE, in the concentration indicated in the legends to the figures and the table, usually for 5 or 10 min. In incubations with [^{3}H]NE, the buffer also contained 10^{-3} M sodium ascorbate. Immediately following this, each culture was rapidly washed five times with 0.75 ml of ice-cold buffer and extracted for 0.5 hr with 0.2 ml of acidic ethanol (95% ethanol:5% 0.1 N HCl). The acidic ethanol was transferred to a scintillation vial, the well washed with another 0.25 ml of acidic ethanol, and radioactivity in the combined acidic ethanol aliquots determined by liquid scintillation spectrometry. In some experiments, cultures were incubated with [^{3}H]*l*-propranolol or [^{3}H]NE in the presence of drugs such as desipramine or *d*-propranolol. In experiments testing the Na^+-dependence of [^{3}H]*l*-propranolol or [^{3}H]NE uptake, 125 mM NaCl in the buffer was replaced by 125 mM LiCl, and 25 mM Tris was used to buffer both the Na^+-containing control buffer and the Na^+-free buffer.

[^{3}*H*]*l-Propranolol and* [^{3}*H*]*NE release.* Release of [^{3}H]*l*-propranolol or [^{3}H]NE was measured from cultures preloaded with [^{3}H]*l*-propranolol or [^{3}H]NE, respectively, as follows. For [^{3}H]*l*-propranolol loading, each culture was washed three times with buffer, incubated for 15 min at 23° with 0.25 ml of 1.4×10^{-7} M [^{3}H]*l*-propranolol and then rapidly washed five times with 0.75 ml buffer at 23°. Cultures were loaded with [^{3}H]NE (10^{-7} M) as described in detail previously [21]. [^{3}H]NE and endogenous catecholamines in chromaffin cell cultures have been shown previously to behave similarly in terms of their release in response to secretagogues, their time courses of release and subcellular storage sites [22]. Thus, it appears that release of [^{3}H]NE accurately reflects endogenous catecholamine release in this system.

Immediately following loading, release of [^{3}H]*l*-propranolol or [^{3}H]NE was measured as follows. Each culture was washed once with 0.5 ml buffer and was incubated for 5 min with buffer alone to determine basal release followed by another 5-min incubation with buffer plus secretagogue to determine stimulated release. Release was measured by determining radioactivity in media from release incubations. At the end of the experiment, each culture was extracted with acidic ethanol, as described above, to determine residual cellular stores of radioactivity. In experiments testing inhibition of [^{3}H]*l*-propranolol or [^{3}H]NE release by drugs such as tetrodotoxin or propranolol, the drug was present for both the 5-min period during which basal release was measured and the 5-min period when stimulated release was measured. In experiments testing the effects of veratridine on [^{3}H]*l*-propranolol release, veratridine was dissolved by acidifying the buffer with 1.0 N HCl. Since acid buffer alone was found

Table 1. Uptake of [^{3}H]*l*-propranolol by chromaffin cell cultures

[^{3}H]*l*-Propranolol (M)	Uptake (pmoles [^{3}H]*l*-propranolol/10^6 cells/5 min)
0.88×10^{-7}	15.5 ± 0.2
1.38×10^{-7}	24.2 ± 1.2
1.04×10^{-6}	170.6 ± 3.1
10^{-5}	1,910.1 ± 148.7
10^{-4}	11,341.6 ± 838.6
10^{-3}	26,598.0 ± 1,060.4

[^{3}H]*l*-Propranolol accumulation by the collagen-coated culture dish alone in the absence of cells was <2% of the accumulation in the presence of cells. Results are the mean ± S.E.M. from four cultures at each concentration of [^{3}H]*l*-propranolol.

to release [^{3}H]*l*-propranolol from chromaffin cells, it was important to bring the pH of the buffer back to 7.4 after dissolving veratridine.

RESULTS

Propranolol uptake. Initial experiments examined some of the characteristics of [^{3}H]*l*-propranolol uptake by cultured chomaffin cells. Table 1 shows the uptake of [^{3}H]*l*-propranolol as a function of the [^{3}H]*l*-propranolol concentration in the medium. [^{3}H] *l*-Propranolol uptake was detectable at $<10^{-7}$ M and increased with increasing concentrations of [^{3}H]*l*-propranolol up to 10^{-3} M; [^{3}H]*l*-propranolol uptake was not saturable within this concentration range. In a concentration range of 5×10^{-7} M to 10^{-3} M, the *d*- and *l*-isomers of propranolol were approximately equipotent in inhibiting [^{3}H]*l*-propranolol uptake (Fig. 1).

Figure 2 compares the time courses of [^{3}H]*l*-propranolol and [^{3}H]NE uptake by chromaffin cell cultures. Uptake of both [^{3}H]*l*-propranolol and [^{3}H]NE were non-linear and the cultures accumulated [^{3}H]*l*-propranolol much more rapidly than [^{3}H]NE. The absolute magnitude of [^{3}H]*l*-propranolol uptake, expressed as moles/10^6 cells/min, was about 44- to 50-fold greater than the uptake of [^{3}H]NE in these cultures incubated with equimolar concentrations of either [^{3}H]*l*-propranolol or [^{3}H]NE.

Cultured bovine adrenal chromaffin cells take up [^{3}H]NE by a high-affinity mechanism that is characterized by (1) its inhibition by low concentrations of desipramine, and (2) its Na^+ dependence [23]. To test if [^{3}H]*l*-propranolol was taken up by this mechanism, I examined the effects of desipramine and Na^+ omission on [^{3}H]*l*-propranolol uptake by chromaffin cell cultures. Figure 3b shows that desipramine, in concentrations from 10^{-7} M to 10^{-5} M, effectively inhibited [^{3}H]NE uptake by 60–75%. However, concentrations of desipramine up to 10^{-6} M were ineffective in inhibiting [^{3}H]*l*-propranolol uptake and 10^{-5} M desipramine inhibited [^{3}H]*l*-propranolol uptake by only 20% (Fig. 3a). Similarly, replacement of 125 mM NaCl in the extra-

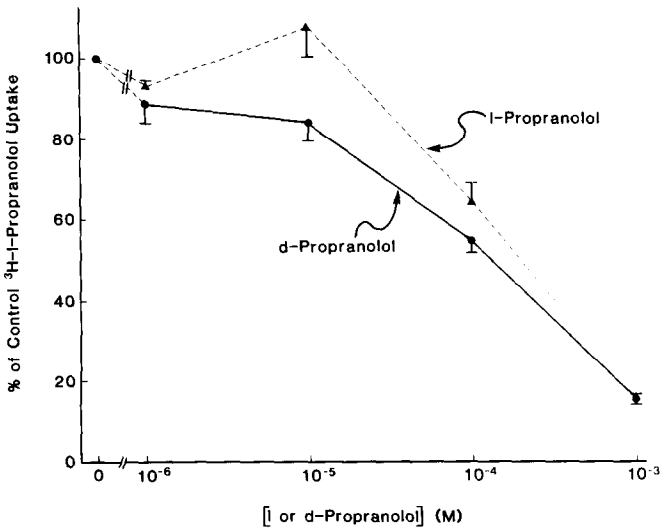

Fig. 1. Inhibition of [^{3}H]*l*-propranolol uptake into chromaffin cell cultures by *l*-propranolol and by *d*-propranolol. Chromaffin cell cell cultures were incubated for 5 min with 10^{-7} M [^{3}H]*l*-propranolol and the indicated molar (M) concentration of *l*- or *d*-propranolol, and [^{3}H]*l*-propranolol uptake was measured. [^{3}H]*l*-Propranolol uptake in the absence of added non-radioactive *l*- or *d*-propranolol was 16.42 ± 0.62 pmoles/10^6 cells/5 min (mean ± S.E.M. from eight cultures). Each point represents the mean ± S.E.M. of results from four cultures.

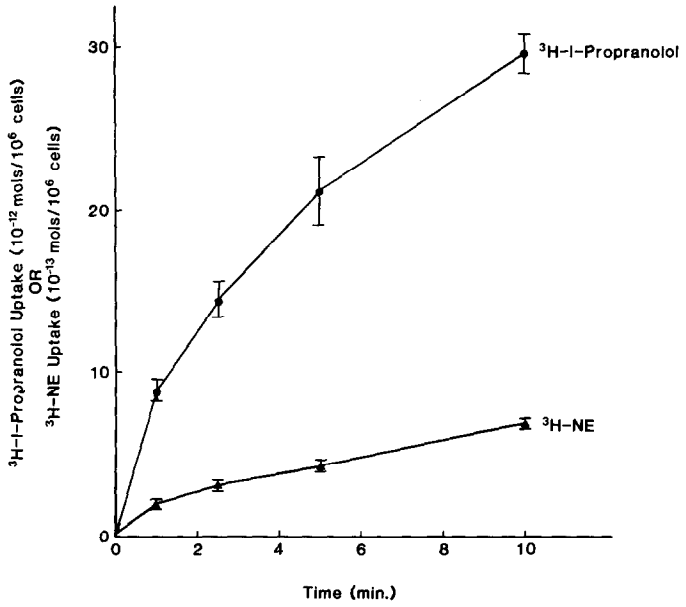

Fig. 2. Time course of [^{3}H]*l*-propranolol and [^{3}H]NE uptake by chromaffin cell cultures. Chromaffin cell cultures were incubated with [^{3}H]*l*-propranolol (10^{-7} M) or [^{3}H]NE (10^{-7} M) for the indicated time period, and [^{3}H]*l*-propranolol or [^{3}H]NE uptake was measured. Note that the unit of [^{3}H]*l*-propranolol uptake is ten times greater than that of [^{3}H]NE uptake. Each point represents the mean ± S.E.M. of results from three cultures.

cellular medium with LiCl reduced [^{3}H]NE uptake by about 55% (Fig. 3b); however, Na^+ replacement had no effect on [^{3}H]*l*-propranolol uptake (Fig. 3a).

Propranolol release. Various agents are known to evoke release of catecholamines from adrenal chromaffin cells. Some of these agents were tested for their ability to release [^{3}H]*l*-propranolol from chromaffin cell cultures, pre-loaded with [^{3}H]*l*-propranolol. Release of [^{3}H]*l*-propranolol was stimulated by two agents, veratridine (Fig. 4a) and tyramine (Fig. 5a), in a dose-dependent manner. Veratridine is known to depolarize cells by activating voltage-sensitive Na^+ channels. However, veratridine-induced [^{3}H]*l*-propranolol release was only slightly inhibited by the Na^+ channel blocker tetrodotoxin (Fig. 4b). The ability of tyramine to release catecholamines is known to be blocked by desipramine. Tyramine-induced [^{3}H]propranolol release from chromaffin cells was not inhibited by desipramine; in fact, desipramine (10^{-6} M to 10^{-5} M) enhanced tyramine-induced [^{3}H]*l*-propranolol release (Fig. 5b). In addition, the following agents failed to evoke release of [^{3}H]*l*-propranolol: a depolarizing concentration of K^+, a cholinergic agonist, carbachol and the physiological mediator of catecholamine release from chromaffin cells, ACh (Fig. 6).

Effects of propranolol on [^{3}H]NE uptake. To determine if propranolol might affect the turnover of catecholamines in chromaffin cells, I tested the effects of propranolol on [^{3}H]NE uptake and release in chromaffin cell cultures. *l*-Propranolol inhibited the uptake of [^{3}H]NE (Fig. 7). *l*-Propranolol was somewhat more potent for this effect at 37° than at 23° at the higher drug concentrations tested (5×10^{-5} M and greater). At 37°, [^{3}H]NE uptake was inhibited almost completely by 5×10^{-4} M propranolol, and the IC_{50} of *l*-propranolol for inhibition of [^{3}H]NE uptake was 5×10^{-6} M. *d*-Propranolol also inhibited [^{3}H]NE uptake; however, the *d*-isomer was somewhat less potent than the *l*-isomer. The highest concentrations of *d*-propranolol tested (5×10^{-5} M and 5×10^{-4} M) produced a maximal inhibition of [^{3}H]NE uptake of about 40% at 23°, while the same concentrations of *l*-propranolol inhibited [^{3}H]NE uptake by about 70% at 23°.

Effects of propranolol on [^{3}H]NE release. By themselves, neither *l*- or *d*-propranolol (10^{-7} M to 10^{-4} M) had any significant effect on basal [^{3}H]NE release, except for the highest concentration of *l*-propranolol (10^{-4} M) which inhibited [^{3}H]NE release by 21.5 ± 6.7% (mean ± S.E.M. from eight cultures). In contrast, both *l*- and *d*-propranolol inhibited the release of [^{3}H]NE from chromaffin cells stimulated by carbachol (Fig. 8). At the higher propranolol concentrations tested (5×10^{-6} M to 10^{-4} M), *l*- and *d*-propranolol were approximately equipotent in inhibiting carbachol-stimulated [^{3}H]NE release. However, there was some stereoselectivity for this effect at the lower propranolol concentrations tested. *l*-Propranolol (5×10^{-7} M and 10^{-6} M) inhibited carbachol-stimulated [^{3}H]NE release by about 45%, while *d*-propranolol in the same concentrations had no effect on carbachol-stimulated [^{3}H]NE release.

DISCUSSION

In the present study, cultures of isolated adrenal chromaffin cells took up [^{3}H]*l*-propranolol by a process that was not saturable in the concentration range of 10^{-7} to 10^{-3} M. Basing calculations on an estimated volume of the cat chromaffin cell [24], the cell

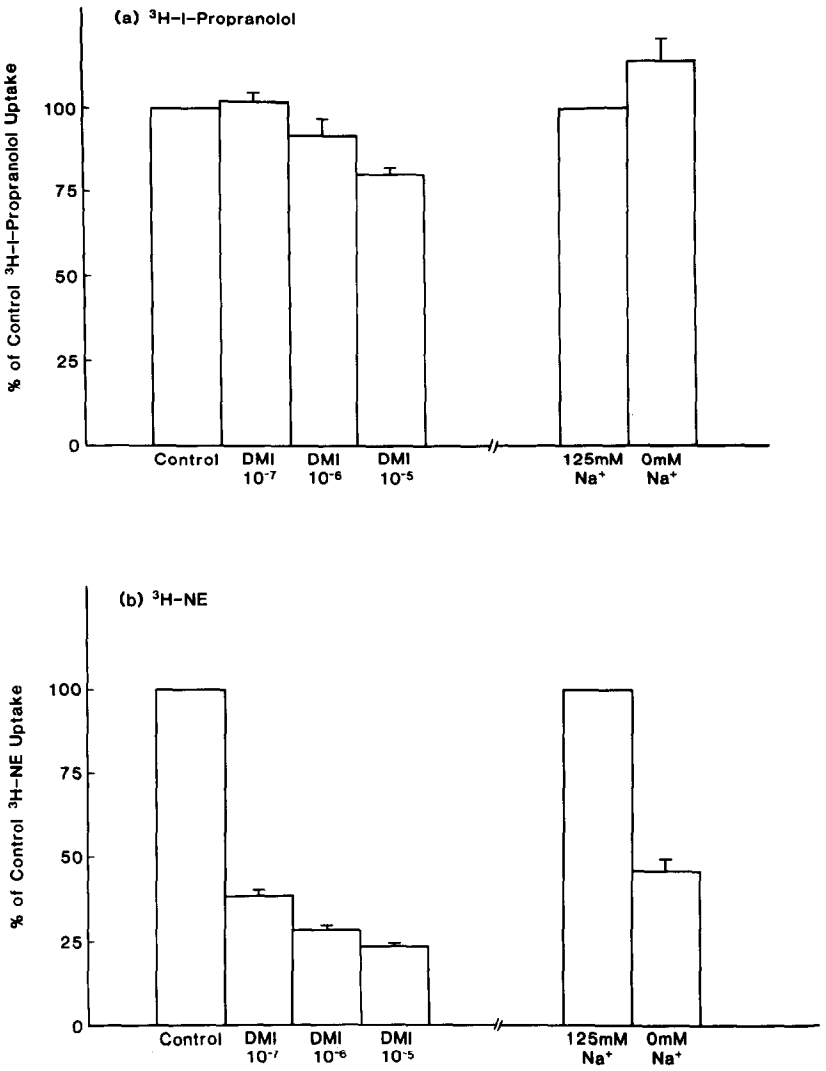

Fig. 3. Effects of desipramine and Na^+ omission on (a) [^{3}H]*l*-propranolol and (b) [^{3}H]NE uptake by chromaffin cell cultures. In experiments testing the effects of desipramine, chromaffin cell cultures were incubated for 10 min with [^{3}H]*l*-propranolol (10^{-7} M) or [^{3}H]NE (10^{-7} M) and the indicated molar (M) concentration of desipramine (DMI). Following this, [^{3}H]*l*-propranolol or [^{3}H]NE uptake was measured. Control [^{3}H]*l*-propranolol uptake, in the absence of desipramine, was 20.88 ± 1.87 pmoles/10^6 cells/10 min and control [^{3}H]NE uptake was 0.90 ± 0.12 pmole/10^6 cells/10 min. Results are the mean ± S.E.M. from three cultures at each concentration of desipramine. In experiments testing the effects of Na^+ omission, chromaffin cell cultures were preincubated for 20 min in buffer containing 125 mM Na^+ or in Na^+-free buffer. The cultures were then incubated for 10 min with [^{3}H]*l*-propranolol (10^{-7} M) or [^{3}H]NE (10^{-7} M) in Na^+-containing or Na^+-free buffer, and [^{3}H]*l*-propranolol or [^{3}H]NE uptake was measured. Control [^{3}H]*l*-propranolol uptake in the 125 mM Na^+ buffer was 13.52 ± 0.80 pmoles/10^6 cells/10 min and control [^{3}H]NE uptake in the 125 mM Na^+ buffer was 0.68 ± 0.02 pmole/10^6 cells/10 min. Results are the mean ± S.E.M. from five to six cultures.

concentration of [^{3}H]*l*-propranolol was approximately 240 times greater than the external medium concentration, following 5 min of incubation with 10^{-7} M [^{3}H]*l*-propranolol. The almost identical inhibition of [^{3}H]*l*-propranolol uptake by increasing concentrations of either *l*- or *d*-propranolol indicates that uptake of propranolol by these cells is probably not stereoselective. Several differences between the mechanisms responsible for uptake of [^{3}H]*l*-propranolol and of [^{3}H]NE were observed. On a molar basis, the magnitude of [^{3}H]*l*-propranolol uptake was much greater (44- to 50-fold) than [^{3}H]NE uptake at early, non-equilibrium, time periods. As has been reported by Kenigsberg and Trifaró [23], [^{3}H]NE uptake by cultured chromaffin cells was partially Na^+ dependent and inhibited by low concentrations of desipramine. However, neither Na^+ omission nor desipramine (10^{-7} M to 10^{-6} M) had any effect on [^{3}H]propranolol uptake. Thus, propranolol does not appear to be transported via the high-affinity uptake system for catecholamines in adrenal chromaffin cells. Experiments with the isolated vas deferens

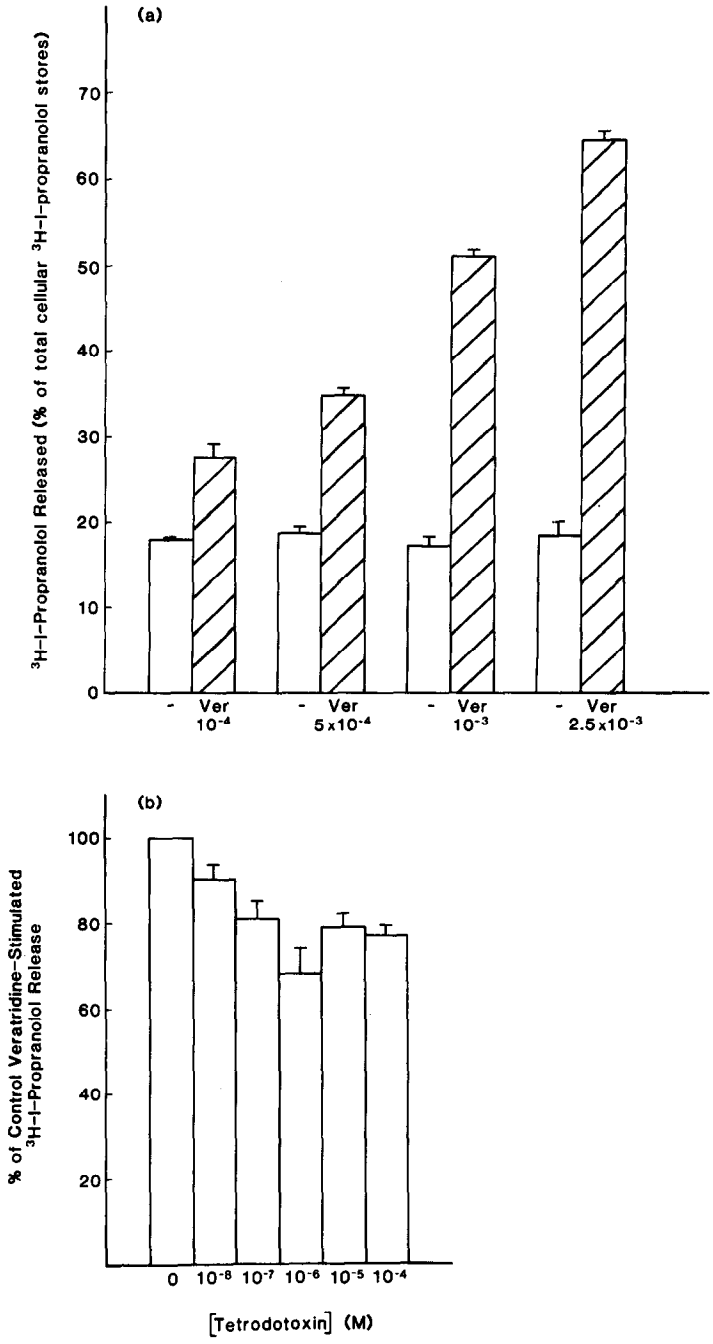

Fig. 4. (a) Effects of veratridine on [^{3}H]*l*-propranolol release from chromaffin cell cultures. Chromaffin cell cultures, preloaded with [^{3}H]*l*-propranolol, were incubated with buffer for 5 min to determine basal [^{3}H]*l*-propranolol release (open bars) followed by a 5-min incubation with the indicated molar (M) concentration of veratridine (Ver., hatched bars), and [^{3}H]*l*-propranolol released into the medium was measured. Results are the mean ± S.E.M. from three cultures at each concentration of veratridine. **(b)** Effects of tetrodotoxin on veratridine-induced release of [^{3}H]*l*-propranolol from chromaffin cell cultures. Chromaffin cell cultures, preloaded with [^{3}H]*l*-propranolol, were incubated for 5 min with the indicated molar (M) concentration of tetrodotoxin to determine basal [^{3}H]*l*-propranolol release. Tetrodotoxin alone had no effect on basal [^{3}H]*l*-propranolol release. Following this, cultures were incubated for 5 min with veratridine (10^{-3} M) and the same concentration of tetrodotoxin to determine the effect of tetrodotoxin on veratridine-induced [^{3}H]*l*-propranolol release. Basal [^{3}H]*l*-propranolol release was subtracted from the total [^{3}H]*l*-propranolol released in the presence of veratridine, to calculate a corrected value for veratridine-induced [^{3}H]*l*-propranolol release. Results are the mean ± S.E.M. from four cultures at each concentration of tetrodotoxin.

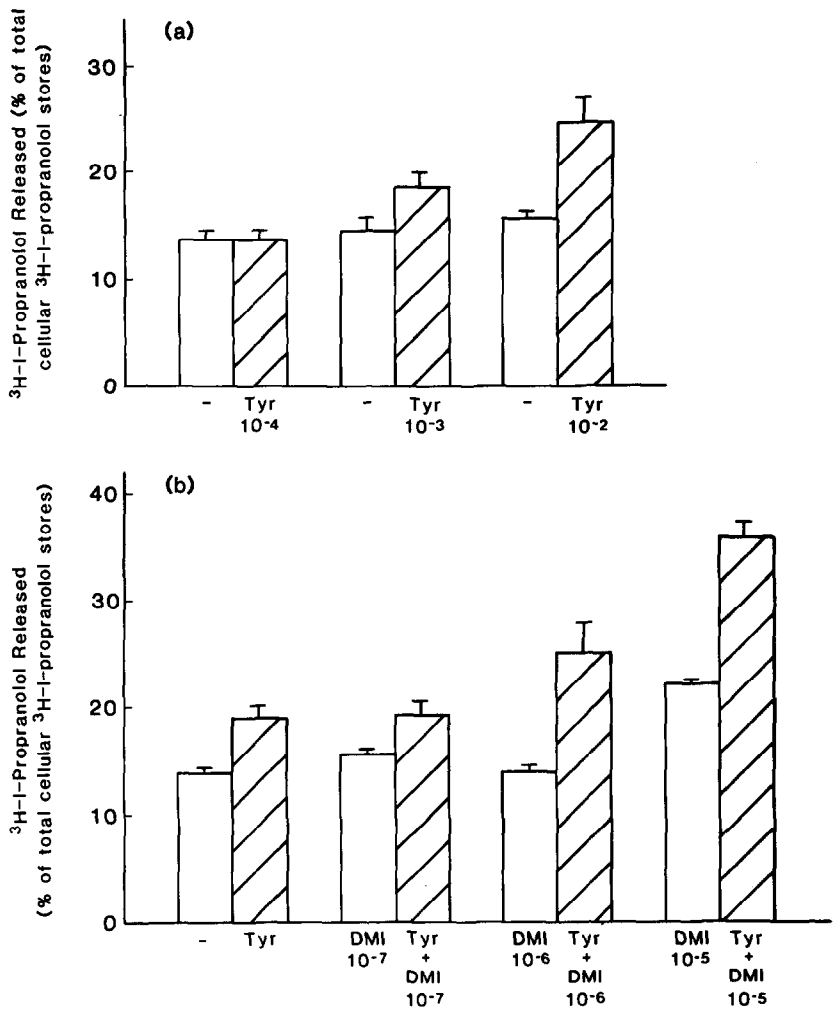

Fig. 5. (a) Effects of tyramine on [^{3}H]*l*-propranolol release from chromaffin cell cultures. Chromaffin cell cultures, preloaded with [^{3}H]*l*-propranolol, were incubated with buffer for 5 min to determine basal [^{3}H]*l*-propranolol release (open bars), followed by a 5-min incubation with the indicated molar (M) concentration of tyramine (Tyr., hatched bars), and [^{3}H]*l*-propranolol released into the medium was measured. Results are the mean ± S.E.M. from four to six cultures at each concentration of tyramine. (b) Effects of desipramine on tyramine-induced release of [^{3}H]*l*-propranolol from chromaffin cell cultures. Chromaffin cell cultures, preloaded with [^{3}H]*l*-propranolol, were incubated for 5 min with the indicated molar (M) concentration of desipramine (DMI) to determine basal [^{3}H]*l*-propranolol release (open bars). Following this, cultures were incubated for 5 min with tyramine (10^{-2} M) and the same concentration of desipramine to determine the effect of desipramine on tyramine-induced [^{3}H]*l*-propranolol release (hatched bars). Results are the mean ± S.E.M. from four cultures at each concentration of desipramine.

[25], cultured sympathetic ganglia [7] and cerebral cortex synaptosomes [3] indicate that accumulation of propranolol by these tissues is also via a mechanism other than the biogenic amine transport system.

Two agents, veratridine and tyramine, were found to stimulate the release of [^{3}H]*l*-propranolol from chromaffin cells preloaded with [^{3}H]*l*-propranolol. Veratridine is known to release catecholamines from chromaffin and other cell types by activating voltage-sensitive Na^+ channels and depolarizing the cells; veratridine-induced catecholamine release is inhibited almost completely by 5×10^{-7} M of the potent Na^+ channel blocker tetrodotoxin [26]. However, 10^{-7} M to 10^{-4} M tetrodotoxin produced only a small inhibition of veratridine-induced [^{3}H]*l*-propranolol release from chromaffin cells. Tyramine-induced catecholamine release is blocked by desipramine, since tyramine must be transported via the biogenic amine uptake mechanism in order to displace catecholamines from their intracellular stores [27]. Desipramine, in concentrations that significantly inhibited [^{3}H]NE uptake into chromaffin cells, did not inhibit tyramine-induced [^{3}H]*l*-propranolol release from chromaffin cells. Therefore these results suggest that the mechanisms by which veratridine and tyramine release [^{3}H]*l*-propranolol from chromaffin cells are different from the mech-

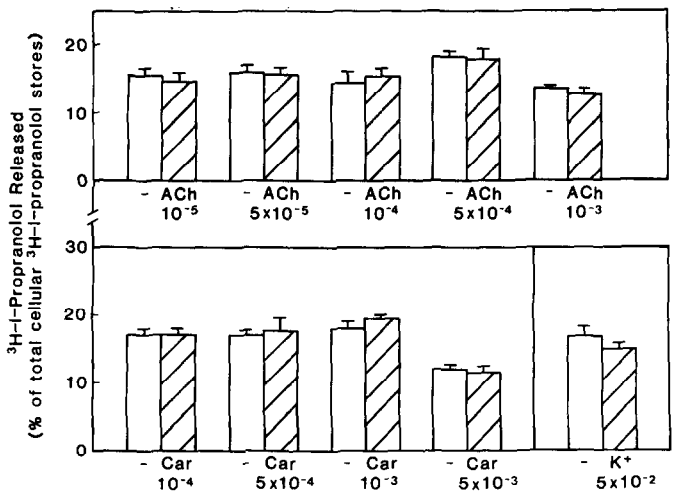

Fig. 6. Effects of acetylcholine, carbachol and K^+ on [^{3}H]*l*-propranolol release from chromaffin cell cultures. Chromaffin cell cultures, preloaded with [^{3}H]*l*-propranolol, were incubated with buffer for 5 min to determine basal [^{3}H]*l*-propranolol release (open bars), followed by a 5-min incubation with the indicated molar (M) concentration of acetylcholine (ACh), carbachol (Car.) or K^+ (hatched bars), and [^{3}H]*l*-propranolol released into the medium was measured. Results are the mean ± S.E.M. from three to six cultures for each concentration of secretagogue.

anisms by which these agents release catecholamines from these cells.

Direct depolarization of the cells with K^+ or stimulation with the cholinergic agonist, carbachol, or the physiological mediator of adrenal catecholamine release, ACh, all failed to evoke release of [^{3}H]*l*-propranolol from chromaffin cells. Thus, it is unlikely that release of propranolol from adrenal chromaffin cells is increased in response to splanchnic nerve stimulation in animals administered propranolol *in vivo*.

Several different modes of stimulation have been

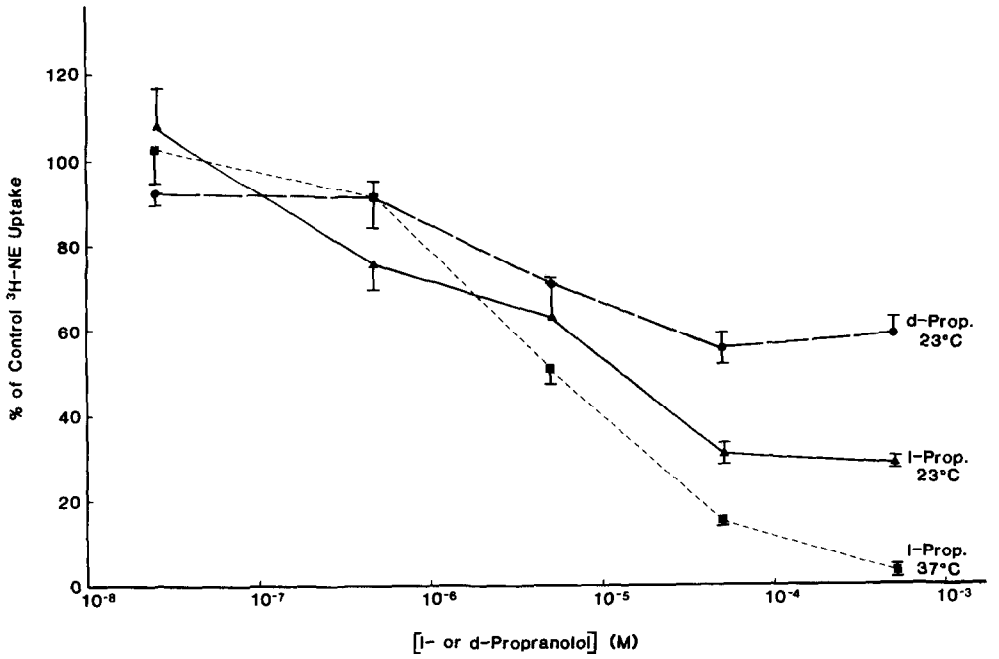

Fig. 7. Inhibition of [^{3}H]NE uptake into chromaffin cell cultures by *l*-propranolol and by *d*-propranolol. Chromaffin cell cultures were incubated for 5 min at 23° or 15 min at 37° with [^{3}H]NE (10^{-7} M) and the indicated molar (M) concentration of *l*- or *d*-propranolol, and [^{3}H]*l*-propranolol uptake was measured. [^{3}H]NE uptake in the absence of propranolol was 0.40 ± 0.02 pmole/10^6 cells/5 min at 23° and 0.75 ± 0.10 pmole/10^6 cells/5 min at 37°. Each point represents the mean ± S.E.M. of results from three to four cultures.

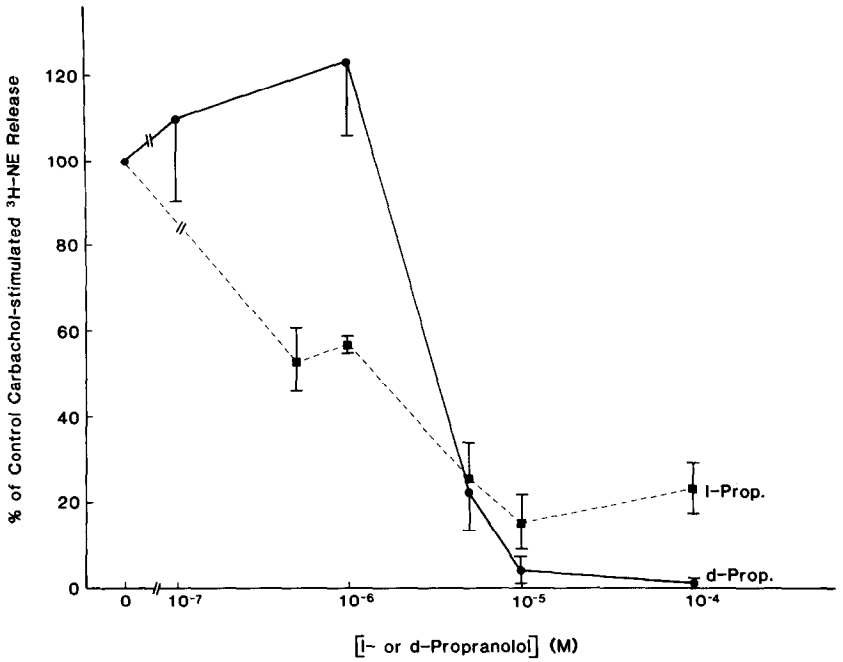

Fig. 8. Inhibition of carbachol-induced [^{3}H]NE release from chromaffin cell cultures by *l*-propranolol and by *d*-propranolol. Chromaffin cell cultures, preloaded for 2 hr with [^{3}H]NE, were incubated for 5 min with the indicated molar (M) concentration of *l*-propranolol or *d*-propranolol to determine the effects of propranolol on basal [^{3}H]NE release. Following this, cultures were incubated for 5 min with carbachol (5×10^{-4} M) and the same concentration of *l*- or *d*-propranolol to determine the effects of propranolol on carbachol-induced [^{3}H]NE release. Basal [^{3}H]NE release was subtracted from the total [^{3}H]NE released in the presence of carbachol to calculate a corrected value for carbachol-induced [^{3}H] NE release. Control carbachol-induced [^{3}H]NE release, in the absence of propranolol, was 5.5 ± 0.8% of total cellular [^{3}H]NE stores. Each point represents the mean ± S.E.M. of results from four cultures.

used in experiments demonstrating stimulated release of propranolol from nervous tissue. These include direct electrical stimulation of nerves to the heart [1], hindlimb and spleen [2], veratridine and/or tyramine stimulation of the heart, cultured sympathetic ganglia [1] and brain synaptosomes [3], and K^+ stimulation of brain synaptosomes [3]. As noted by Daniell *et al.* [1], direct electrical stimulation of nerves may displace the lipophilic propranolol molecule from membrane-bound sites by disrupting those membranes. In addition, Lewis [25] could detect no increased release of radiolabelled propranolol, proctolol or penbutolol during electrical stimulation of the vas deferens, preloaded with these drugs. In experiments using veratridine and tyramine, blockade of propranolol release by tetrodotoxin and desipramine, respectively, has not been reported; thus, it is unknown if the mechanisms by which these drugs release propranolol from nerve endings are similar to their neurotransmitter releasing mechanisms. Finally, while K^+ depolarization did elicit release of [^{3}H]propranolol from brain synaptosomes in the experiments of Street *et al.* [3], only 37% of K^+-induced [^{3}H]propranolol release was Ca^+ dependent compared to 91% Ca^{2+} dependence for K^+-induced [^{3}H]NE release. Thus, it is unclear, at this point, whether accumulated propranolol is released from noradrenergic nerve endings by stimulation of these nerves under physiological conditions.

In the present study, *l*-propranolol inhibited [^{3}H]NE uptake by chromaffin cell cultures with an IC_{50} of 5×10^{-6} M at 37°. However, *l*-propranolol was at least two orders of magnitude less potent than desipramine in inhibiting [^{3}H]NE uptake. At 23°, 10^{-5} M propranolol inhibited [^{3}H]NE uptake by 50% compared to a greater than 50% inhibition of [^{3}H]NE uptake by 10^{-7} M desipramine. Both the *l*- and *d*-forms of propranolol inhibited [^{3}H]NE uptake by chromaffin cells, although the *d*-isomer was somewhat less potent than the *l*- at higher propranolol concentrations (5×10^{-5} M, 5×10^{-4} M). Since the *l*-isomer of propranolol is generally much more potent than the *d*-form as a β-adrenoceptor antagonist, propranolol's inhibition of [^{3}H]NE uptake into chromaffin cells is probably not mediated by a β-adrenoceptor. In the heart and in cerebral cortex synaptosomes, inhibition of [^{3}H]NE uptake by propranolol and other β-adrenoceptor antagonists is also unrelated to the β-adrenoceptor blocking properties of these compounds [11, 14] but may be correlated to their lipophilicity [14].

Propranolol, in both the *d*- and *l*-forms, in concentrations up to 10^{-4} M, did not directly release [^{3}H]NE from isolated adrenal chromaffin cells in culture. Thus, contrary to the suggestion of Sugawara *et al.* [17], propranolol probably does not directly release catecholamines from the adrenal medulla *in vivo*. However, experimental evidence does suggest that propranolol can release catecholamines from sympathetic adrenergic nerves. For example, Daniell

et al. [10] have reported that injections of propranolol into the coronary artery of the dog cause an increased release of NE from the heart, and Saelens *et al.* [7] have shown that propranolol increases the release of [^{3}H]NE from vasa deferentia preloaded with the radiolabelled amine. Propranolol has also been reported to release [^{3}H]NE from cerebral cortical slices; however, a relatively high concentration (10^{-3} M) of propranolol was required for this effect [13].

l-Propranolol inhibited carbachol-induced [^{3}H]NE release from cultured adrenal chromaffin cells. This is in agreement with the results of Greenberg and Zinder [18] who demonstrated that racemic *d,l*-propranolol inhibits ACh-stimulated catecholamine release from freshly isolated chromaffin cells. In the present study, stimulation-induced [^{3}H]NE release was reduced by about 45% by 5×10^{-7} M *l*-propranolol, the lowest concentration tested. At 5×10^{-7} M and 10^{-6} M *l*-propranolol, this effect was stereoselective since *d*-propranolol in the same concentrations did not inhibit carbachol-induced [^{3}H]NE release. Therefore, at these lower propranolol concentrations, the inhibition by propranolol of stimulation-induced [^{3}H]NE release may be mediated via the β-adrenoceptor. This is consistent with the suggestions of Boonyaviroj and Gutman [28] and Greenberg and Zinder [18] that β-adrenoceptors may be involved in the regulatory fine control of catecholamine release from adrenal chromaffin cells. At higher concentrations (5×10^{-6} M to 10^{-4} M), *l*- and *d*-propranolol were approximately equipotent in inhibiting carbachol-induced [^{3}H]NE release from cultured chromaffin cells; thus, these effects were probably not due to β-adrenoceptor blockade but may be related to the membrane-stabilizing properties of propranolol.

Propranolol is widely used therapeutically in the treatment of hypertension and part of the hypotensive effect of the drug may be related to its effects on catecholamines in the adrenal. Several studies have provided evidence for a role of adrenomedullary catecholamines in the control of blood pressure, particularly in the hypertensive state [29–31], and significant increases in plasma epinephrine (E) [32–38] and NE [32, 34, 37, 39, 40] have been observed in human subjects with hypertension. In this context, it is of interest to note that Cousineau *et al.* [34] and Kuchel *et al.* [38] have identified subpopulations of patients with labile hypertension, in whom an increase in circulating catecholamines could be accounted for mainly by an increase in plasma E (i.e. from the adrenal).

The reported effects of β-adrenoceptor blocking drugs, such as propranolol, on plasma catecholamines and blood pressure are complex. In the rat an acute pressor effect is seen with intravenous administration of propranolol to normotensive [41–43] or hypertensive [44] animals and this increase in blood pressure is associated with an increase in adrenal venous E and NE levels [17]. However, acute intraperitoneal administration of optimal doses of propranolol to the conscious rat does produce hypotension [43]. Most studies report that, with more acute administration in man, β-adrenoceptor blocking agents increase plasma E and NE levels in both normotensive [16, 45, 46] and hypertensive [15] subjects. However, the hypotensive effects of β-adrenoceptor blockers in man are generally seen only with chronic drug treatment [47], and after prolonged (4 month) treatment with the β-adrenoceptor antagonist prindolol, Brecht *et al.* [48] reported a decrease in plasma NE levels in patients with essential hypertension. Adler-Graschinsky and Langer [4] have suggested previously that blockade of prejunctional β-adrenoceptors by propranolol and a subsequent decrease of NE release from sympathetic nerve endings may contribute to the antihypertensive actions of propranolol (and other β-adrenoceptor blockers). Consistent with this idea is the observation of Cousineau *et al.* [34] that propranolol is more effective in lowering blood pressure in a population of hypertensives with increased plasma NE than in those with normal plasma NE; the effects of propranolol in hypertensives with increased plasma E were not reported in this study. However, it is possible that the ability of propranolol to directly inhibit stimulation-induced catecholamine release from adrenal chromaffin cells may also contribute to the anti-hypertensive effects of propranolol in some patients.

In conclusion, adrenal chromaffin cells accumulate propranolol; however, stimulation probably does not increase the efflux of propranolol from the cells. Propranolol does not appear to release catecholamines from adrenal chromaffin cells. Propranolol does inhibit stimulation-induced catecholamine release from chromaffin cells at lower propranolol concentrations and inhibits catecholamine uptake at higher concentrations. These actions of propranolol may affect catecholamine levels in the blood and could conceivably account for a part of the hypotensive effect of propranolol.

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Note added in proof: Bright *et al.* have reported recently that veratridine-induced release of [^{3}H]propranolol from rat cerebral cortex synaptosomes is partially inhibited by tetrodotoxin [P. S. Bright, T. E. Gaffney, J. A. Street and J. G. Webb, *Br. J. Pharmac.* **84**, 499 (1985)].

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