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STEREOSELECTIVE DELIVERY AND ACTIONS OF BETA RECEPTOR ANTAGONISTS

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Abstract—These studies have revealed that the delivery and actions of beta receptor antagonist drugs are controlled by a cascade of stereoselective processes involving multiple enzymes, transport proteins and receptors. In essence, the free concentration of the pharmacologically active (−)-enantiomer species of these drugs presented to cell surface beta receptors appears to be a function of the stereoselective clearance by hepatic cytochrome P-450 isoenzymes, enantiomer selective binding to α_1-acid glycoprotein and albumin and perhaps predominantly by stereoselective sequestration (and release) by the vesicular amine transport protein within adrenergic neurons.

Stereoselectivity in the clearance of beta blocking drugs, which can favor either the (+)- or (−)-enantiomer, only appears to be important for the lipophilic drugs which are cleared by hepatic metabolism. Such stereoselectivity is due to differential stereochemical substrate requirements of individual hepatic cytochrome P-450 isoenzymes. Interindividual variations in the stereoselectivity can occur as a result of differences in the amount and expression of cytochrome P-450 isoenzymes due to genetic predisposition or other factors. In the same context, we have observed a significant correlation between the extent and stereoselectivity of binding of beta blocking drugs to plasma proteins. This is another finding which suggests that variability in the expression of individual proteins involved in the beta blocking drug-protein cascade determines the free concentration of the pharmacologically active enantiomer. However, since most observations have been made in young normal subjects, the extent of stereoselectivity in metabolism, binding and other processes is unknown in the general population where steady-state plasma concentrations can vary widely due to multiple biological factors.

The observations from neural studies support the concept that adrenergic nerve endings provide a depot for the stereoselective storage and release of the active enantiomer of beta receptor antagonists. The mechanism of this release appears to involve exocytotic secretion of drug that has been stereoselectively accumulated by the neurotransmitter storage vesicles. In terms of this idea, beta receptor antagonists released during nerve stimulation may achieve concentrations of the (−)-enantiomer within the adrenergic synapse greatly in excess of those found in plasma. Such a mechanism could significantly influence both the intensity and duration of beta receptor blockade in the heart, blood vessels, brain and other target tissues. Indeed, persistence of the cardiovascular effects of beta receptor antagonists after plasma levels of drug have apparently declined below effective concentrations could be explained in part by this phenomenon. In any event, it seems clear that stereoselective storage and secretion by nerve endings provides an additional mechanism for modulating the delivery and concentration of the active enantiomer of beta receptor blocking drugs at sites of action within the adrenergic synapse.

In conclusion, multiple enzymes, transport proteins and other proteins, each with specific stereochemical requirements, determine the delivery and therapeutic actions of beta receptor antagonist drugs.

Beta receptor blocking drugs were designed to antagonize the binding and therefore the physiological actions of the neurotransmitter norepinephrine at the beta adrenergic receptor. Since beta adrenergic receptor binding for the natural neurotransmitter norepinephrine is stereoselective for the (−)-enantiomer, it is perhaps not surprising that beta receptor blocking drugs show analogous stereoselectivity. This has been shown to be the case for all beta blocking drugs, although the stereoselectivity for a given antagonist, depending upon its structure, can vary widely with a (−)/(+) enantiomer ratio for the binding affinity to the beta receptor being as low as 10 for atenolol and as high as 1000 for pindolol [1].

The principal therapeutic effects of the beta receptor blocking drugs are also stereoselective for the (−)-enantiomer. Thus, the antianginal effects are clearly mediated via cardiac beta receptor blockade and should predictably involve the (−)-enantiomer. Further, even for therapeutic actions where the mechanism is less clearly understood, such as in the management of hypertension, the (−)-enantiomer appears to represent the active form of the drug. Thus in hypertensive patients, in which daily doses

of 120 to 320 mg of (±)-propranolol decreased supine systolic and diastolic as well as standing systolic blood pressure, Rahn *et al.* [2] demonstrated that the same doses of (+)-propranolol had no effect on blood pressure.

Considering the enantiomeric selectivity associated with beta receptor binding and the therapeutic actions of beta receptor antagonists, it is of practical concern that almost all of these drugs are currently administered as their racemates, i.e. a 50:50 mixture of the (−)- and (+)-enantiomers. Because of the highly variable asymmetric nature of the biological environment to which these drugs are exposed, it is very possible that the enantiomeric composition of the beta blocking drugs is dramatically altered at their sites of action. Therapeutic drug level monitoring and pharmacokinetics have been extensively used in an effort to optimize therapy with beta receptor blocking drugs. However, until recently, measurements of these drugs in plasma, tissue and other body fluids have not distinguished between the (−)-, or active, and the (+)-enantiomer. Thus the true levels of the pharmacologically active species of receptor antagonist drugs have been unknown and our understanding of the relationships between the plasma and tissue concentrations and therapeutic effects of these drugs has been incomplete.

Separation and quantitation of individual enantiomers in biological samples after administration of racemic drugs is no longer an insurmountable problem. Extensive research in analytical chemistry, in particular over the last several years, has provided many approaches. It is the purpose of this paper to discuss findings with respect to the stereochemistry of the delivery and actions of beta blocking drugs.

This paper will more specifically focus on the stereochemistry of the hepatic metabolism and plasma binding of beta blocking drugs, emphasizing observations on propranolol and other lipophilic agents. As very large species differences in the stereoselectivity of the metabolism of beta blocking drugs exist [3], observations will be limited to findings in man. We will also address a stereoselective transport process that appears to be involved in the enantiomer-selective delivery of beta blocking drugs to their sites of action in blood vessels, heart, brain, and other tissues, namely, the stereoselective sequestration and release of the (−)-enantiomer of beta receptor antagonist drugs by adrenergic neurons.

STEREOSELECTIVE METABOLISM AND DISTRIBUTION OF BETA RECEPTOR ANTAGONISTS

Several studies using different analytical methodology for the separation of the enantiomers have examined the stereochemistry of the oral clearance of propranolol in man [4–7]. The data summarized in Table 1 express the (−)/(+) enantiomer ratios for the plasma propranolol concentrations observed in 42 normal subjects. The (−)-enantiomer concentrations exceeded those of the (+)-enantiomer by 40–90% as an average. It can also be seen from these data that the interindividual variability in the (−)/(+) enantiomer ratio is quite large, varying about 4-fold from 0.99, i.e. demonstrating no stereoselectivity, to a value of 3.71. The conclusion is that there is a clear stereoselectivity in the oral clearance of this drug in most individuals, with preferential removal of the (+)-enantiomer from the circulation.

As stereoselective hepatic metabolism is the most likely mechanism for these enantiomeric differences in oral clearance, it becomes important to consider the contribution of individual metabolic pathways to these differences. Although numerous metabolites are formed *in vivo* in man [8], all metabolic products can be attributed to three primary pathways, i.e. glucuronidation, side-chain oxidation and ring oxidation. The oxidative pathways appear to be mediated by distinct cytochrome P-450 isoenzymes [9, 10]. Whereas propranolol glucuronide represents the glucuronidation pathway, naphthoxylactic acid, formed by dealkylation, deamination and oxidation of an intermediate aldehyde, has been shown to reflect total side-chain oxidation [11] (Fig. 1). Ring oxidation is more complex. After initial 3,4-epoxidation, the 4-hydroxypropranolol formed is almost completely conjugated with glucuronic acid and sulfate. Both conjugation pathways appear to be stereoselective in man with glucuronic acid conjugation favoring the (−)-enantiomer [7, 12] and sulfoconjugation favoring the (+)-enantiomer [7, 12, 13]. Determination of the stereoselectivity of cytochrome P-450 mediated ring oxidation therefore requires measurements of the enantiomers of both 4-hydroxypropranolol conjugates.

A preliminary study, determining the stereochemical composition of metabolites in the urine of normal subjects, suggested stereoselective ring oxidation [12]. A more comprehensive study was

Table 1. Plasma propranolol enantiomer levels in normal subjects receiving oral doses of racemic drug

(−)/(+)-Ratio of Plasma Levels				
Mean	Range	Dosage	Number studied	References
1.48*	0.99–2.04	Single 40 mg	15	[4]
1.87†	1.07–3.71	Repeated 40–80 mg	4 × 3 doses	[5]
1.55†	1.39–1.74	Single 80 mg	6 × 2 doses	[6]
1.39†	1.08–1.78	Single 80 mg	17	[7]

* Plasma enantiomer levels at 2 hr after dose.
† Area under the plasma concentration–time curves, i.e. complete kinetics.

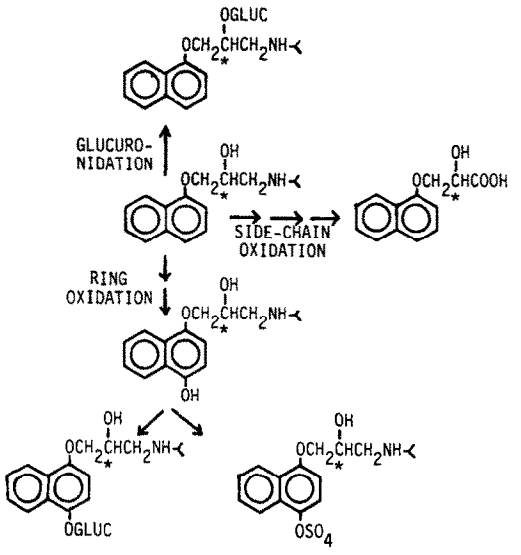

Fig. 1. Partial metabolic scheme for propranolol in man [8].

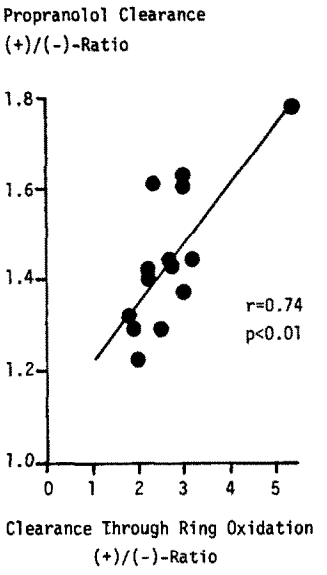

Fig. 2. Relationship between the stereoselectivity of propranolol oral clearance and the stereoselectivity of ring oxidation clearance in 14 normal subjects after an 80 mg single oral dose [7].

recently completed in 17 subjects (see group 4 in Table 1). The oral clearance of (+)- and (−)-propranolol was obtained from plasma concentration measurements of the enantiomers, whereas the partial clearances of the enantiomers through the three metabolic pathways in Fig. 1 were obtained from urinary excretion data. Determination of partial metabolic clearances is a way to monitor the *in vivo* activity of the hepatic enzymes involved in propranolol metabolism [11, 14]. As shown in Table 2 the higher clearance of (+)- as compared to (−)-propranolol in the 17 subjects studied was solely due to a 2.5-fold greater clearance of the (+)- as compared to the (−)-enantiomer through ring oxidation. The clearance through both glucuronidation and side-chain oxidation was identical for the (+)- and (−)-enantiomers. Thus, the reason for enantiomeric differences in propranolol's oral clearance appears to involve differences in the catalytic activities of one or more cytochrome P-450 isoenzyme(s) involved in the ring oxidation of (+)- and (−)-propranolol.

As shown in Table 1 there was a large inter-individual variability in the stereoselectivity of propranolol oral clearance. The nature of this variability was examined in these same 17 subjects where the (−)/(+) enantiomer ratio of plasma propranolol concentrations ranged from 1.08 to 1.78. Three of these subjects had very low ratios (1.08, 1.08 and 1.15), indicating very little stereoselectivity. These three individuals also had by far the lowest clearance through the stereoselective metabolic step, i.e. ring oxidation (0.13 ± 0.11 l/min compared to 0.72 ± 0.45 l/min for the rest of the group). It is also interesting to note that two of these individuals were poor oxidizers of debrisoquine, a genetic trait shown to be associated with suppression but not abolition of ring oxidation of propranolol [7, 15, 16]. Thus, as might be expected, individuals who show no stereoselectivity in the oral clearance of propranolol are those in whom the stereoselective pathway is a minor contributor to hepatic metabolism and clearance of the drug. In the remaining 14 subjects there was a clear association between the stereoselectivity of

Table 2. Propranolol enantiomer clearances and the partial metabolic clearances (l/min) through glucuronidation (propranolol glucuronide), side-chain oxidation (naphthoxylactic acid) and ring oxidation (4-hydroxypropranolol glucuronide + sulfate) of the enantiomers after single oral racemic doses in 17 subjects [7]

Propanolol		Propranolol glucuronide		Naphthoxy-lactic acid		4-Hydroxy-propranolol glucuronide + sulfate	
(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)
2.78	1.96	0.24	0.27	0.38	0.31	0.88	0.35
±1.36	±0.87	±0.10	±0.12	±0.28	±0.10	±0.70	±0.30
$P < 0.001$		NS		NS		$P < 0.001$	

propranolol's oral clearance and the stereoselectivity of ring oxidation (Fig. 2). The stereoselectivity of ring oxidation varied as much as 3-fold, with (+)/(−) enantiomer ratios ranging from 1.8 to 5.4. The basis for this variability is unclear but may be due to varying amounts of multiple cytochrome P-450 isoenzymes catalyzing this reaction, each with different stereochemical requirement. We have previously observed a similar phenomenon in the stereoselectivity of hepatic sulfoconjugation of 4-hydroxypropranolol [13]. From previous observations in man it appears clear that ring oxidation of propranolol is catalyzed by at least two isoenzymes, one being the debrisoquine isoenzyme [7, 15, 16], the other(s) as yet unidentified.

The therapeutic importance of stereoselective oral clearance of propranolol has not been directly assessed, although it is apparent that measurements of racemic drug in plasma to varying degrees will underestimate the circulating levels of active drug ((−)-enantiomer). In a study by Coltart and Shand [17] it was shown that racemic propranolol was two- to three-fold more potent after oral than after intravenous doses at equal plasma concentrations. This interesting observation might to a large extent be explained by the findings above of a greater clearance of (+)-propranolol after oral as opposed to intravenous doses (see further below), although a contributing factor could also be the higher concentrations of the active metabolite 4-hydroxypropranolol after oral than after intravenous doses [18].

Our knowledge of the stereochemistry of the oral clearance of other beta blocking drugs is more limited; however, examination of the reported observations adds significantly to our understanding of the stereochemistry of the metabolism and actions of this class of drugs.

Single oral doses of metoprolol have been shown in one study [19] to undergo stereoselective oral clearance similar to propranolol, producing plasma concentrations with a (−)/(+) enantiomer ratio of 1.37 with a range of 1.09–1.86. This enantio-selectivity has been proposed to be due to preferential *O*-demethylation of the (+)-enantiomer [20], the major metabolic pathway [21] (Fig. 3). α-Hydroxylation of metoprolol, a minor pathway [21], is reportedly selective for the (−)-enantiomer [20], and should have the opposite effect on the clearance of the enantiomers of metoprolol.The stereoselectivity in oral clearance of metoprolol appeared to be higher in subjects with high total clearance. In these subjects the drug is cleared faster through the predominant stereoselective metabolic pathway. The metabolism of metoprolol is more sensitive than propranolol to

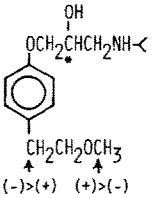

Fig. 3. Molecular sites and proposed stereoselectivity of metoprolol oxidation [20].

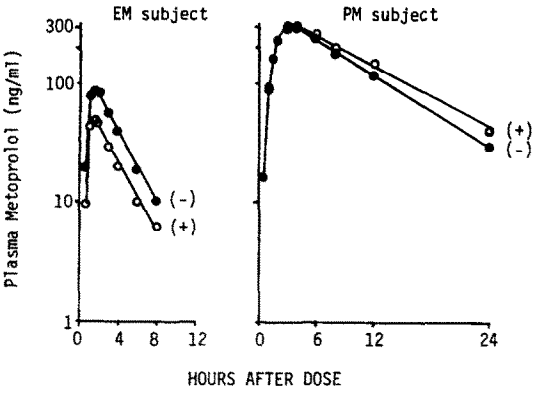

Fig. 4. Plasma concentration–time curves for the metoprolol enantiomers after 200 mg oral doses of racemic drug in one extensive metabolizer (EM) and one poor metabolizer (PM) of debrisoquine [19].

the debrisoquine-type of genetic deficiency. Metoprolol oral clearance is markedly reduced in subjects with this genetic trait [19] and the stereoselectivity of the oral clearance is completely abolished in such individuals (Fig. 4). Since this drug in poor metabolizers has a low hepatic extraction ratio, the half-lives of both enantiomers are also prolonged [19]. Thus, the stereoselectivity of the oral clearance and actions of metoprolol appears to be highly dependent on the debrisoquine polymorphism status. An interesting drug interaction with quinidine was recently described [22]. This drug, which effectively inhibits the debrisoquine isoenzyme *in vitro*, clearly inhibited the oral clearance of metoprolol. As in poor metabolizers of debrisoquine, quinidine abolished the stereoselectivity of metoprolol clearance.

Several other beta blockers have also been shown to undergo stereoselective oral clearance. These drugs include bufuralol [23, 24], penbutolol [25] and xibenolol [26]. Their chemical structures are shown in Fig. 5 with arrows indicating the sites and proposed stereoselectivities of metabolic hydroxylations. For bufuralol and penbutolol, plasma concentrations after single oral doses showed mean (−)/(+) enantiomer ratios of 1.97 and 2.68, respectively, i.e. similar to propranolol and metoprolol. However, for xibenolol this ratio was only 0.43. This drug thus demonstrates opposite stereoselectivity compared to the other beta blockers, i.e. the active (−)-enantiomer is cleared more rapidly than the (+)-enantiomer. This may appear to be a peculiar observation. However, as seen with metoprolol and in particular bufuralol, cytochrome P-450 mediated oxidations have different preferences for the individual enantiomers dependent on the site of hydroxylation (Figs 3 and 5). Our limited knowledge of the structural requirements for cytochrome P-450 oxidations together with the multiple isoenzymes with overlapping substrate specificities do not permit predictions of the stereochemistry of these reactions.

Two additional points are worth making with respect to these drugs. First, bufuralol metabolism like metoprolol metabolism is highly sensitive to the debrisoquine polymorphism status. Thus, in poor metabolizers of debrisoquine both oxidative pathways are greatly suppressed. However, in contrast

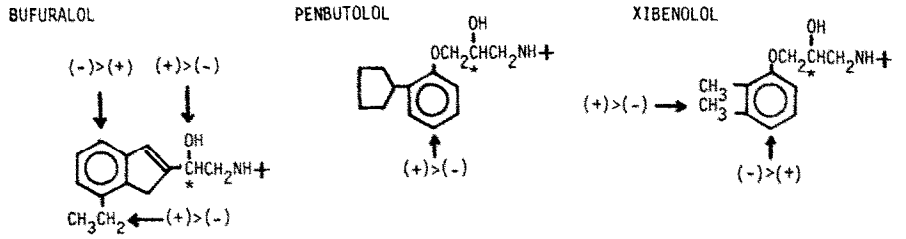

Fig. 5. Molecular sites and proposed stereoselectivity of the metabolism of bufuralol [20, 23, 24, 27, 28], penbutolol [25] and xibenolol [26].

to metoprolol, the stereoselectivity in total oral clearance of bufuralol is not abolished but rather enhanced in these subjects, (−)/(+) enantiomer ratio in plasma of 2.54 [23]. This was proposed to be due to stereoselective glucuronidation of (+)-bufuralol. This appears to be the only beta blocker for which stereoselective glucuronidation of the beta hydroxyl group has been observed in man. Second, xibenolol produces two metabolites, a 4-hydroxy- and a 3-hydroxymethyl-metabolite, which are 2–3 times more potent than the parent drug as beta blockers [25]. The plasma concentrations of these metabolites, in particular those of the (−)-enantiomer of the 4-hydroxy metabolite, are much higher than those of the parent drug, suggesting this as the main pharmacologically active form of the drug. This is the only beta blocker studied so far in man where most of the biologic activity may reside in a metabolite.

The observations made with respect to the molecular site(s) of the stereoselectivity of beta-blocking drug metabolism in man have mostly been made *in vivo*. A seemingly more direct approach is to use hepatic microsomes or reconstituted human cytochromes P-450. This approach has generated valuable insights into the stereochemistry of metabolism of metoprolol [20] and in particular bufuralol [20, 27, 28] (see Figs 3 and 5). However, there are also discrepancies between *in vitro* [29, 30] and *in vivo* data which are not understood, although excessive substrate concentrations *in vitro* can lead to saturation phenomena and altered stereoselective metabolism.

Differences in the plasma concentrations of the enantiomers of propranolol and other beta blockers could also at least in part be due to stereoselectivity in drug distribution. To investigate this possibility we undertook several studies to examine the intravenous dose kinetics and blood binding of the enantiomers of propranolol in normal subjects. A summary of these observations is given in Table 3. A number of parameters demonstrated rather small but significant enantiomeric differences. The systemic clearance was higher for (+)-propranolol due to stereoselective hepatic metabolism [31]. The small enantiomeric difference when compared to the oral clearance discussed above is predictable. For a high hepatic extraction drug like propranolol the full effect of the intrinsic hepatic clearance is seen only after oral doses [32]. After intravenous doses the delivery of the drug from tissue binding sites to the liver, i.e. hepatic blood flow, will mask differences in the intrinsic ability of the liver to extract and remove the enantiomers. The volume of distribution, i.e. extravascular distribution, was also higher for (+)-propranolol, which may be due to stereoselectivity in blood or tissue binding or both. The half-lives of the enantiomers were identical, probably reflecting the opposing effects of clearance and volume of distribution on this pharmacokinetic parameter, i.e. a higher clearance for (+)-propranolol would tend to decrease half-life for this enantiomer and a larger volume of distribution of this enantiomer would tend to increase half-life. Of particular interest was the finding of stereoselective plasma binding of (−)-propranolol [33], since this process has not been

Table 3. Intravenous pharmacokinetics and blood binding characteristics for propranolol enantiomers [33, 35]

Parameter*	(+)-P	(−)-P	(+)-P/(−)-P
Cl_s, l/min	1.21 ± 0.15	1.03 ± 0.12†	1.17 ± 0.02
V_d, l/kg	4.82 ± 0.34	4.08 ± 0.33†	1.18 ± 0.02
$t_{\frac{1}{2}}$, hr	3.57 ± 0.25	3.53 ± 0.21	1.01 ± 0.02
$f_{unbound}$	0.203 ± 0.008	0.176 ± 0.007†	1.15 ± 0.01
$V_d$unbound, l/kg	20.3 ± 1.7	18.9 ± 1.7†	1.07 ± 0.02
α_1-$AGP_{unbound}$	0.162 ± 0.017	0.127 ± 0.013†	1.28 ± 0.01
$HSA_{unbound}$	0.607 ± 0.013	0.649 ± 0.011†	0.94 ± 0.01
RBC/$plasma_{unbound}$	3.16 ± 0.24	3.05 ± 0.28	1.04 ± 0.01

* Abbreviations used: (+)-P, (+)-propranolol; (−)-P, (−)-propranolol; Cl_s, systemic clearance; V_d, volume of distribution; $t_{\frac{1}{2}}$, half-life; $f_{unbound}$, unbound fraction in plasma; $V_d$unbound, volume of distribution of unbound drug; α_1-$AGP_{unbound}$, fraction not bound to α_1-acid glycoprotein; $HSA_{unbound}$, fraction not bound to human serum albumin; RBC/$plasma_{unbound}$, red blood cell concentration to unbound plasma concentration.

† Significantly different from (+)-P, $P < 0.05$ to 0.001.

considered stereoselective for basic drugs. This selectivity could account for virtually all of the enantiomeric difference in the volume of distribution of propranolol. To characterize further the plasma binding difference, propranolol binding to the major plasma binding proteins for basic drugs, i.e. α_1-acid glycoprotein (α_1-AGP) and human serum albumin (HSA), was investigated [33]. This clearly demonstrated (Table 3) that the enantioselective plasma binding was due to stereoselective binding of (−)-propranolol to α_1-AGP. It was also interesting to note the very slight but significant opposite stereoselectivity in the binding of racemic propranolol to HSA. This emphasizes the highly variable asymmetric nature of biological macromolecules with which drugs and other chemicals interact. Although these stereoselective distributional differences for propranolol do not appear large enough to be of therapeutic significance, it is of interest that the enantioselectivity of plasma binding increases with greater total binding. This is shown in Fig. 6, which includes observations made both in man (low binding) [33] and dog (higher binding) [34]. Thus, in subjects with unusually high binding of propranolol such as in conditions of elevated α_1-AGP levels, e.g. myocardial infarction [35] as well as other disease conditions [36, 37], the unbound fraction of active (−)-propranolol may decrease, thereby limiting the access of this enantiomer to beta receptors and other target proteins.

The stereoselectivities observed in hepatic metabolism and plasma and non-specific tissue binding involve only the most lipophilic of the beta blocking drugs. The hydrophilic drugs, e.g. atenolol, are not metabolized, and are not extensively bound to either plasma or tissue proteins. Their elimination from the body is by renal mechanism only. A slight stereoselectivity in the renal clearance of two beta blockers with intermediate lipophilicity, i.e. metoprolol [19] and pindolol [38], has been observed. Whether the enantiomers of the hydrophilic beta blocking drugs are handled differently by the kidneys and whether this will influence their clearance is not known.

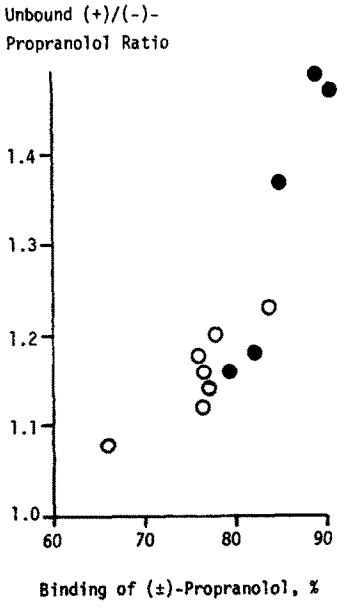

Fig. 6. Relationship of unbound (+)/(−)-propranolol ratio to the total (±)-propranolol plasma binding in man (○) [33] and dog (●) [34].

STEREOSELECTIVE STORAGE AND RELEASE OF BETA RECEPTOR ANTAGONISTS BY ADRENERGIC NEURONS

An additional mechanism for regulating the delivery and disposition of the active (−)-enantiomer of beta receptor blocking drugs at target sites in a variety of tissues has been revealed in our studies of the interactions of beta receptor antagonists with adrenergic nerve endings. Results from experiments in animals, man and *in vitro* models support the idea that propranolol and other beta adrenergic receptor blocking drugs are candidates for stereoselective storage and calcium-dependent release by adrenergic nerve endings. In *in situ* experiments with dogs pretreated orally with propranolol for seven days, direct (electrical) and indirect (tyramine-induced) stimulation of the cardiac accelerator nerves has been shown to produce a parallel release of propranolol along with norepinephrine from nerve endings in the heart [39] (Figs 7 and 8). Propranolol release in response to sympathetic nerve stimulation was also reported in blood vessels of the perfused canine hindlimb and in the perfused spleen where venous-arterial differences of drug increased by as much as 100 ng/ml during sympathetic nerve stimulation [40]. Similarly, activation of the sympathetic nervous system in man by graded treadmill exercise was found to produce a stepwise increase in plasma levels of propranolol in treated subjects with a time course that closely paralleled changes in plasma norepinephrine [41]. Finally, neural release of the cardioselective beta receptor antagonist atenolol (Fig. 9) as well as propranolol has been demonstrated *in vitro* using rat brain synaptosomes as a model [42, 43]. In these studies membrane depolarization with either potassium or veratridine was shown to stimulate the efflux of both propranolol and atenolol from cortical synaptosomes and the effect of veratridine to augment drug release was antagonized by tetrodotoxin. Thus, propranolol release has been observed during sympathetic nerve stimulation at a variety of adrenergic neuroeffector sites *in situ*, during exercise stress in man and from isolated preparations of nerve endings. The demonstration of similar release of atenolol indicates that hydrophilic beta receptor antagonists have the same potential to be stored and secreted by adrenergic nerve endings and suggests, further, that this phenomenon may be generally applicable to beta receptor blocking drugs as a class.

To examine the mechanisms of neural storage and release of beta receptor blocking drugs and to test for stereoselectivity in these processes, studies of atenolol release have been conducted using the PC12 clonal line of rat pheochromocytoma cells as a model neurosecretory system [44]. PC12 cells were loaded by incubation overnight with racemic (±)-[^{3}H]atenolol and then subjected to a washout protocol in the presence and absence of depolarizing concentrations of extracellular potassium. Samples

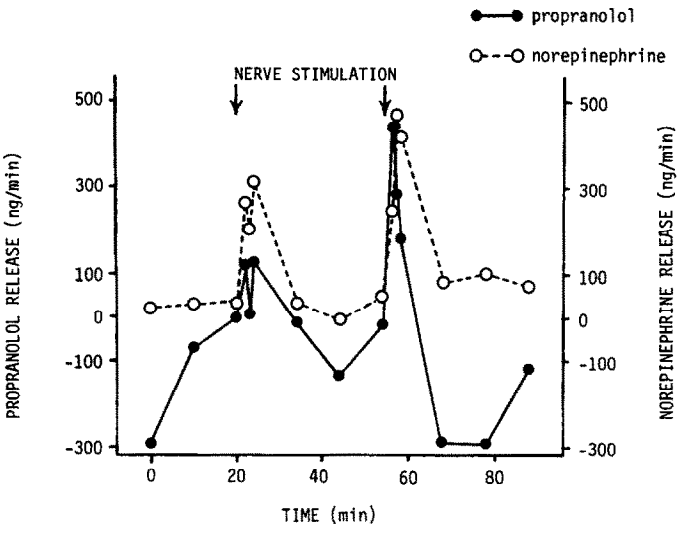

Fig. 7. Propranolol and norepinephrine release from the canine heart during stimulation of the cardiac accelerator nerves [39].

of radiolabeled atenolol released from these cells into the incubation buffer under varying experimental conditions were then resolved into the individual (+)- and (−)-enantiomers using chiral derivatization and subsequent separation of the diastereomers using reverse phase HPLC. [^{3}H]Atenolol was found to be rapidly secreted by preloaded PC12 cells in response to membrane depolarization, i.e. high potassium (Table 4), and secretion was completely dependent upon the presence of extracellular calcium [45]. During passive efflux of atenolol in the presence of 5 mM extracellular potassium, the ratio of (−)/(+) atenolol isomers released from the PC12 cells was approximately 1.7:1 (Table 4). In comparison, when potassium was elevated to 50 mM the ratio of (−)/(+) atenolol released into the medium was increased to a value of 3.6:1. These results support the existence within PC12 cells of stereoselective and calcium-dependent mechanisms for atenolol release which result in preferential secretion of the (−)-enantiomer of the drug in response to neural activation.

When uptake of atenolol by PC12 cells was examined, uptake across the cell membrane of intact cells was found to be slow, not readily saturable and not influenced by amine transport inhibitors. Conversely, uptake of atenolol by isolated neurotransmitter storage vesicles from these cells showed

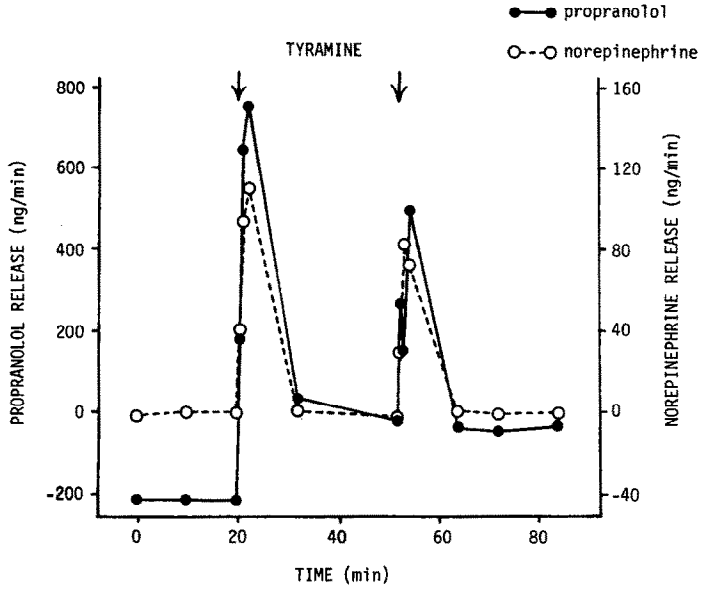

Fig. 8. Propranolol and norepinephrine release from the canine heart after the administration of two bolus injections of tyramine [39].

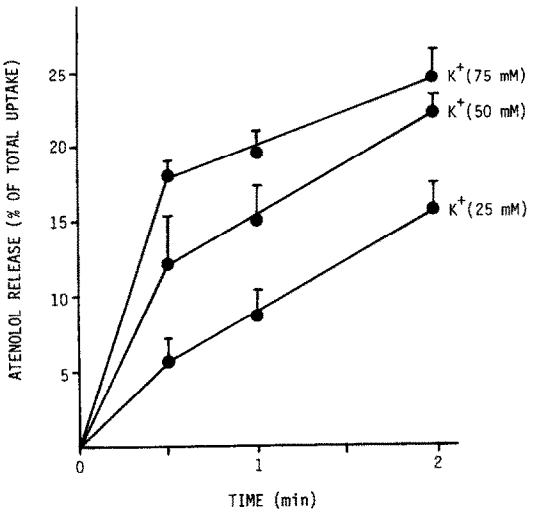

Fig. 9. Potassium-induced release of [^{3}H]atenolol from rat cortical synaptosomes [43].

a significant active component with uptake in the presence of ATP being approximately 6-fold greater than that observed in the absence of ATP (Table 5). In addition, this uptake was markedly reduced at 0° and blocked by reserpine (IC_{50} = 12 nM) and inhibited in a competitive manner by the (−)-enantiomer of norepinephrine. Active transport of atenolol into the storage vesicles was also found to be stereoselective [46] (Table 5). At 37° in the presence of 1 mM ATP, uptake of (−)-atenolol by vesicles incubated with racemic drug was 5-fold greater than uptake of (+)-atenolol. A very similar stereoselectivity in favor of the (−)-enantiomer has been observed for the vesicular uptake of norepinephrine [47]. In comparison, the small amount of atenolol passively accumulated by the storage vesicles at 0° or in the presence of reserpine showed a predictable (−)/(+) enantiomer ratio of 1. These data indicate that the active beta blocking enantiomer of atenolol is selectively transported into adrenergic storage vesicles by the biogenic amine carrier protein. Presumably, this stereoselective sequestration of drug by the storage vesicles then accounts for the preferential release of (−)-atenolol when PC12 cells are depolarized.

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Table 4. Stereoselective release of atenolol enantiomers from PC12 cells in response to membrane depolarization

Condition	Atenolol release* % Released	Atenolol release* (−)/(+) Enantiomer ratio
5 mM K^+, 2 mM Ca^{2+}	1.0 ± 0.2	1.7 ± 0.1
50 mM K^+, 2 mM Ca^{2+}	7.5 ± 0.9†	3.6 ± 0.2†
50 mM K^+, 0 mM Ca^{2+}	1.5 ± 0.2	

* Release during 5 min of exposure to incubation buffer. Each value represents the mean ± SE of 3–5 determinations.
† Differs significantly from 5 mM K^+, 2 mM Ca^{2+}, $P < 0.05$.

Table 5. Stereoselective uptake of atenolol enantiomers by amine storage granules isolated from PC12 cells

Condition*	Atenolol uptake pmol/mg protein	(−)/(+) Enantiomer ratio
37°	2.20 ± 0.81	5.00 ± 0.44
−ATP	0.385	
0°	0.149 ± 0.048†	1.09 ± 0.05†
Reserpine 1 μM; 37°	0.230	1.05

* ^{3}H-Atenolol—0.2 μM; Mg^{2+},ATP (1 mM); 15 min incubation.
Values = mean ± SE.
† Differs significantly from 37°, $P < 0.05$.

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