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Tissue atenolol levels following chronic β-adrenoceptor blockade using oral atenolol in dogs

M. K. DAVIES*, J. MCAINSH, *Department of Cardiovascular Medicine, University of Birmingham, East Birmingham Hospital, Bordesley Green East, Birmingham, B9 5ST, UK, and International Medical Affairs Department, Imperial Chemical Industries PLC, Alderley Park, Cheshire UK*

Tissue atenolol concentrations are high following chronic continuous β-adrenoceptor blockade in dogs. Furthermore, significant concentrations of this poorly lipid soluble drug are found within the central nervous system after chronic dosing. It is suggested that all β-adrenoceptor blocking agents may enter the central nervous system in significant and sufficient quantities to account for a central antihypertensive action of this group of compounds. Sequestration of β-adrenoceptor agents in the CNS or other tissues may account for other clinically observed effects including adaptive effects.

Despite the widespread use of β-adrenoceptor blocking agents in the treatment of cardiovascular disorders, their mechanisms of action, particularly in hypertension, remain unknown. Proposed mechanisms have included effects on the enzymic activities in sympathetic ganglia (Raine & Chubb 1977) and central nervous system effects (Reid et al 1974; Myers et al 1975). This has led, however, to a number of controversies especially as hydrophilic β-adrenoceptor blocking drugs, which theoretically enter the central nervous system poorly, are still very effective antihypertensive agents (Neil-Dwyer et al 1981). Furthermore, the antihypertensive effects of these drugs may be much delayed following initiation of therapy but, conversely persist for many weeks after withdrawal (Prichard & Gillam 1969; Zacharias & Cowen 1970; Tarazi & Dustan 1972). Finally, the effects of acute β-adrenoceptor blockade on cardiovascular haemodynamics (Prichard & Gillam 1969; Tarazi & Dustan 1972; Brundin et al 1976) and noradrenaline-synthesizing enzymes (Raine & Chubb 1977) in sympathetic ganglia may be substantially different from those observed after chronic therapy.

Atenolol is one of the least lipid soluble β-adrenoceptor blocking agents (Barrett 1977). This study was designed to examine the concentrations of atenolol in the central nervous system following chronic continuous β-adrenoceptor blockade.

Methods

Nine dogs were maintained on chronic oral atenolol therapy, 100 mg twice daily (dosing at 0900 and 1630 h) for a mean period of 98 days (range 21 to 212 days). The effectiveness of this regimen in producing continuous β-adrenoceptor blockade throughout the 24 h period was determined using ambulatory electrocardiographic monitoring and isoprenaline challenge testing. 24 h ambulatory monitoring provided mean hourly heart rate data in 4 dogs in the control period and after stabilization on chronic oral atenolol therapy. Similarly, the chronotropic responses to increasing bolus intravenous injections of isoprenaline were determined in one dog during the control and chronic treatment phases. These methods have been reported elsewhere (Davies et al 1984).

After a mean period of 98 days the dogs were examined 18 h after the last dose of atenolol. Samples of left ventricular myocardium, lung, skeletal muscle, liver, kidney, spleen and cerebral hemispheres were flash frozen in liquid nitrogen within 30 min and stored at −30 °C until required. Serum samples were obtained immediately after death and similarly stored at −30 °C. Serum and tissue atenolol concentrations were determined by the gas liquid chromatographic technique described by Scales & Copsey (1975), which is sensitive to 0·04 μg ml^{-1}. The preparation of plasma and tissue samples using butanol–cyclohexane as solvent was carried out exactly as described by Scales & Copsey (1975). Atenolol concentrations in the various tissues were compared using Student's independent *t*-test.

Results

Using this atenolol dosage regimen, continuous β-adrenoceptor blockade was obtained throughout the 24 h period. Each mean hourly heart rate on oral atenolol therapy was below the level recorded during the control phase (Fig. 1). The reductions at both ends of the 24 h were significant (55·1 beats min^{-1} compared with a control value of 67·8 beats min^{-1} at 0700–0800h, t = 5·02, $P \ll 0{\cdot}001$; at 1000–1100h. 70·8 beats min^{-1} compared with a control of 86·0, t = 4·65, $P \ll 0{\cdot}001$). These results have been reported elsewhere (Davies et al 1984).

The isoprenaline challenge test was carried out in one dog 21 h after the last dose of oral atenolol. The result would therefore reflect less than the minimum degree of β-adrenoceptor blockade occurring during the chronic study as the maximum interval between doses was 17 h. A significant degree of β-adrenoceptor blockade was present at 21 h, with 11·8 μg of isoprenaline kg^{-1} being required to produce a chronotropic response of 50 beats min^{-1} compared with 0·24 μg kg^{-1} before treatment in the same dog (Fig. 2).

* Correspondence.

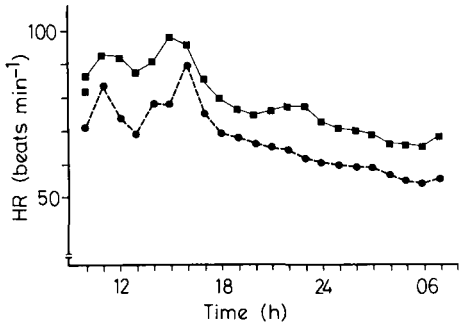

FIG. 1. Reductions in mean hourly heart rates (HR) in four dogs on chronic oral atenolol therapy. All mean hourly heart rates on treatment are below control levels. (■) = control (●) = chronic β-adrenoceptor blockade.

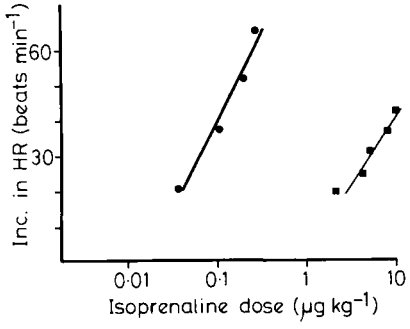

FIG. 2. Isoprenaline challenge test in the same dog before treatment (●) and during chronic oral atenolol therapy (■) 21 hours after the last atenolol dose.

The mean serum atenolol level (±s.d.) 18 h after the last oral dose was 0·64 ± 0·31 μg ml^{-1}. The simultaneous mean tissue atenolol concentrations, in μg g^{-1} wet weight of tissue, are shown in Table 1. The myocardial atenolol concentration was higher than the serum atenolol concentration in every case. The mean myocardial concentration (±s.d.) was approximately double that of the mean serum concentration (myocardium 1·25 ± 0·51 μg g^{-1}; serum 0·64 ± 0·31 μg g^{-1}) and this was a significant difference (t = 3·07, $P < 0·01$). Atenolol concentrations in lung, skeletal muscle and liver were similar (1·8–1·9 μg g^{-1}) and were all significantly higher than in the serum (t = 3·86, t = 3·74, t = 5·11, $P < 0·001$, respectively). The levels within these tissues were also higher than the myocardial concentration but these differences were not significant. The highest concentrations of atenolol were found in the kidney and spleen with mean values of 2·42 and 3·1 μg g^{-1}, respectively. These are 4 to 5-fold higher than the mean serum concentration.

The concentrations of atenolol in the central nervous system were high. The mean (±s.d.) atenolol concentration obtained from samples of cerebral cortex in each case was 0·7 μg g^{-1} which is actually higher than the simultaneous plasma concentration although the difference was not significant (t = 0·29).

Discussion

The mechanism of action of β-adrenoceptor blocking agents remains unknown, despite their widespread use for many years. In particular, postulates regarding a central antihypertensive effect have been controversial as a number of agents having significant antihypertensive effects have low lipid solubilities and would therefore only enter the central nervous system poorly (Neil-Dwyer et al 1981). Atenolol is one of the least lipid soluble of the β-adrenoceptor blocking agents with an octanol–water partition coefficient of 0·23 (Barrett 1977). It has been reported to enter the central nervous system very poorly following acute intravenous dosing, as shown by radioactive labelling of atenolol in rats and assessment of whole body autoradiograms (McAinsh, personal communication) or direct tissue levels (Street et al 1979). These findings cannot be used as evidence against a central mechanism of action since the clinical efficacy of these drugs is often only seen after chronic therapy and the haemodynamic effects following acute and chronic therapy are substantially different (Prichard & Gillam 1969; Zacharias & Cowen 1970; Tarazi & Dustan 1972; Brundin et al 1976). For example the full antihypertensive effects may take many weeks to become apparent. Tissue levels following chronic therapy may therefore be more relevant. The gas chromatographic method used to determine atenolol concentration is sensitive to 0·10 μg ml^{-1} for fluids and 0·04 μg ml^{-1} for tissue (Scales & Copsey 1975). The analytical method is drug-specific and would not detect metabolites of atenolol. Indeed atenolol is metabolized

Table 1. Mean atenolol concentrations (±s.d.)in serum (μg ml^{-1}) and tissue (μg g^{-1}) following chronic β-adrenoceptor blockade.

Dog No	Wt (kg)	Dose (mg kg^{-1} day^{-1})	Treatment (days)	Serum	Heart	Lung	Skeletal muscle	Spleen	Liver	Kidney	Brain
1	27·5	7·3	21	0·28	0·53	0·73	0·78	1·84	1·47	0·81	0·45
2	22·5	8·9	31	0·60	1·58	1·18	1·40	3·02	1·35	2·11	0·61
3	30·5	6·6	26	0·59	2·01	2·00	—	3·10	1·60	3·25	0·85
4	26·5	7·5	33	0·52	1·37	2·68	2·83	4·46	2·13	2·72	0·85
5	23·0	8·7	126	0·54	0·87	2·39	1·14	—	2·83	3·2	—
6	22·5	8·9	157	1·41	1·88	—	3·68	—	—	—	—
7	21·5	9·3	155	0·55	0·71	—	1·98	—	—	—	—
8	27·0	7·4	212	0·68	1·06	—	1·87	—	—	—	—
9	27·0	7·4	117	0·58	1·25	—	1·45	—	—	—	—
Mean	25·3	8	98	0·64	1·25	1·8	1·9	3·1	1·9	2·42	0·7
±s.d.	±2·9	±0·9		±0·31	±0·51	±0·8	±0·95	±1·1	±0·6	±1·0	±0·2

to only a very small degree (McAinsh & Holmes 1983). Furthermore, the most useful information is obtained in studies demonstrating chronic continuous β-adrenoceptor blockade rather than intermittent blockade over long periods of time. In the study reported here, continuous blockade for 24 h was obtained using oral atenolol at 100 mg twice daily. This was evidenced by the significant reductions in the mean hourly heart rates throughout the 24 h and the significant reductions in isoprenaline-induced tachycardia at the end of the longest interval between doses. Isoprenaline challenge tests were not carried out in any of the dogs (numbers 1 to 4) used for the tissue level studies. This was avoided because of the theoretical possibility that isoprenaline might influence the results via displacement of atenolol from the β-receptor. Furthermore, in the other drugs (numbers 5 to 9) none received isoprenaline within a minimum of 6 weeks before plasma/tissue level estimation.

There is a large variation in the length of treatment between the 9 dogs, but, in the four studies of CNS atenolol levels, the mean treatment period was 28 days within a range of 21 to 33 days. After this mean period of 28 days, significant concentrations of atenolol were seen within the CNS with levels similar to those simultaneously found in plasma. Thus CNS penetration does occur in significant quantities after chronic treatment in the dog. This may, in part, explain some of the clinical observations regarding the slow onset of the antihypertensive effect. As atenolol is one of the least lipid soluble β-adrenoceptor blocking agents, it is a reasonable assumption that all such agents enter the CNS in significant quantities thereby supporting the concept of a central mechanism of action. Similar observations have been reported in the rat after the use of radioactively labelled atenolol. Following acute intravenous atenolol, the CNS penetration was poor but was facilitated by chronic dosing—the levels being significantly higher after 3 weeks treatment (Street et al 1979). Studies of CNS concentrations in man are few (Neil-Dwyer et al 1981, Cruickshank et al 1980). In 3 patients receiving oral atenolol for 3–10 days (100 mg once daily), the atenolol brain/plasma ratio was only 0·1 : 1·0, suggesting very poor CNS penetration. However, the duration of treatment in at least 2 of these patients was short and perhaps insufficient to achieve significant CNS levels.

In this study, samples of cerebral cortex were obtained to estimate CNS levels of atenolol. Undoubtedly there will be something detected due to plasma contamination of the tissue which has a blood supply. However, this contribution to the results is small because the relative density of blood vessels is small and the results are expressed as μg atenolol g^{-1} tissue.

The time requirement for significant tissue accumulation may also explain some of the observed findings relating to the clinical effects of these agents. Noradrenaline synthesising enzyme levels in sympathetic ganglia are normal following acute β-adrenoceptor blockade. It is only after 6–12 days treatment that the levels become reduced. This can be explained on the same basis as the findings relating to central nervous system penetration.

It has been shown that the pharmacodynamic effects of atenolol on the reduction in exercise-induced tachycardia are correlated with the plasma drug levels (McAinsh 1977). This is perhaps not the most suitable criterion as tissue levels suggested here may be significantly higher than plasma levels after chronic therapy. In particular the levels in the myocardium were twice those of the plasma. Levels in other tissues were much higher than this (Table 1). These findings can again be correlated with some observed clinical effects. A number of adaptive effects (defined as effects persisting after the withdrawal of therapy and when plasma levels are undetectable by the particular assay procedure) have been reported. These have included effects on haemodynamic parameters (Prichard & Gillam 1969; Tarazi & Dustan 1972; Morgan et al 1975; Brundin et al 1976), electrophysiological measurements (Vaughan Williams et al 1975; Raine & Vaughan Williams 1976; Vaughan Williams 1977), effects on sympathetic nervous system enzymes (Raine & Chubb 1977) and a prolonged myocardial protective effect (Nayler et al 1977). Also, the antihypertensive effect has been found to persist long after drug withdrawal (Zacharias & Cowen 1970; Amery et al 1977). The findings in the present study suggest two possibilities to explain these adaptive effects. Firstly the levels within the CNS may exhibit a similar time course in decay as in accumulation, providing for a prolonged central effect whilst serum levels are below the detectable range or absent. Secondly, the sequestration of the particular agent in tissues such as the heart and skeletal muscle may provide a source of continuing availability of the drug to the body as it is slowly leached out of the tissue.

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Autonomic regulation involved in the ocular hypotensive action of β-adrenergic blocking agents

M. ALKONDON, A. RAY, P. SEN*, *Department of Pharmacology, University College of Medical Sciences, Ring Road, New Delhi 110029, India*

The effects of racemic propranolol and some related drugs on the intraocular pressure (IOP) were studied after topical application in rabbits. These drugs produced a significant reduction in IOP with the following order of potency: (−)-propranolol > timolol > (±)-propranolol > sotalol > (+)-propranolol > pranolium. Pretreatment of rabbit eyes with atropine significantly antagonized the ocular hypotensive action of (±)-propranolol, timolol and pranolium. Both (±)-propranolol and timolol produced a significant increase in pupil diameter in the presence of a submydriatic dose of atropine. The activities of monoamine oxidase and carbonic anhydrase were unaffected by (±)-propranolol, timolol and pranolium in-vitro. It is concluded from the results that both cholinergic and adrenergic mechanisms may be involved in the ocular hypotensive effects of the drugs.

Both timolol and propranolol have been reported to reduce the intraocular pressure (IOP) in rabbits and in glaucoma patients (Radius et al 1978; Vale & Phillips 1970). However, the exact mechanism by which these drugs produce an ocular hypotensive action remains unclear (Chiou 1981). The cholinesterase enzyme inhibitory activities for propranolol and other related drugs have been reported by Alkondon et al (1983). In view of this, we have explored the possible involvement of a cholinergic mechanism in the ocular hypotensive effect of some β-blockers.

Materials and methods

Experiments on rabbits. Albino rabbits of either sex (1·5–2·0 kg), were housed under standard conditions and exposed to a 12 h light-dark cycle. Intraocular pressure was measured by means of a Schioetz tonometer (Medicon Instruments, Germany). A large number of preliminary IOP readings were made after local anaesthesia with 1% lignocaine in order to accommodate the animals to the measurement procedure. However, during the actual experiments, the readings were made after sedating the animals with diazepam (17·5 μmol kg^{-1} i.m.), 30 min before the start of IOP measurements. All drug studies were carried out in a blind fashion by two experimenters, one applying the drug (or vehicle) and the second, who had no knowledge of the schedule of drug administration until the end of the entire study, measuring the IOP. Six rabbits received (in two 50 μl doses, 5 min apart) drug dissolved in phosphate buffered saline, pH 7·4 on one eye and saline (vehicle) on the contralateral eye, into the inferior conjunctival sac. The six test drugs used were coded as D_1 to D_6 and each of the six animals received one of the drugs on day 1. On day 2, the drugs were given (by changing the order) to the eye which received vehicle on the first day. This procedure was repeated till the 6th day by which time, each animal had been exposed to all six drugs. These test drugs were administered in a concentration of 15 mM, since this approximately equals the concentrations of timolol and propranolol used in clinical and experimental situations (i.e. 0·5%).

The IOP measurements were made at 0, 30 60, 90, 120 and 180 min after drug or vehicle administration. In a second group of rabbits, the IOP readings were taken only after application of vehicle on both eyes. In another group, atropine was given 30 min before the test drugs and the IOP measurements observed. In all the animals, the corneal reflex was tested with a cotton swab, whereas the pupil diameter was measured by

* Correspondence.