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ANALYSIS OF THE HYPOTENSIVE EFFECTS OF MEDROXALOL AND ITS ENANTIOMERS, MDL 17,330A AND MDL 17,331A

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ABSTRACT

Medroxalol and two of its enantiomers (MDL 17,330A, MDL 17,331A) were tested for activity at adrenergic receptors to clarify the mechanism(s) of the antihypertensive action of medroxalol. The potency order for blocking postsynaptic α_1-receptors *in vivo* in rats was similar to that reported *in vitro*. However, relatively high doses of these compounds were necessary to block α_1-receptors *in vivo* compared to their antihypertensive doses. Postsynaptic α_2-receptors were not blocked by medroxalol *in vitro*. Propranolol pretreatment of rats to block vascular β_2-receptors antagonized much of the hypotensive response to medroxalol and its enantiomers, most extensively with MDL 17,330A, but much less with MDL 17,331A. The potency rank order as β_2-adrenergic agonists was the same as previously reported for these compounds as β_1-adrenergic receptor antagonists. The stimulation of β_2-adrenergic receptors in vascular smooth muscle appears to be an important factor in the hypotensive action of medroxalol.

INTRODUCTION

Medroxalol is a new antihypertensive agent currently undergoing clinical trials. It is one of a series of compounds reported to be antagonists of α- and β-adrenergic receptors (11). It is apparent from previous reports (11) that there is no simple

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relationship between the antihypertensive actions of medroxalol analogs and their potencies as either α_1 - or β_1-adrenoceptor antagonists *in vitro*. Moreover, the blockade of β-receptors by medroxalol-like compounds probably does not contribute to the immediate blood-pressure lowering effect of these compounds since administration of other drugs with only β-blocking activity does not lower blood pressure acutely (2,10,17). Recently, it has been reported that medroxalol is a partial agonist at β_2-adrenoreceptors and that this effect may contribute to its antihypertensive activity (6,15).

This study defines the effects of medroxalol and enantiomers on adrenergic receptors and their contribution to the hypotensive action of medroxalol. Medroxalol itself has two asymmetric centers and therefore is composed of four enantiomers, in approximately equal proportions. These four compounds are equipotent to medroxalol as hypotensives in the spontaneously hypertensive rat (SHR), although, as with the medroxalol analogs, their hypotensive efficacy does not correlate with the *in vitro* assessment of their α_1 - and β_1-adrenergic blocking potency (4). Two enantiomers were chosen for further evaluation and comparison to medroxalol, namely MDL 17,330A and MDL 17,331A, since, of the four enantiomers, these two possess the greatest contrast in their potencies as adrenoceptor antagonists *in vitro* (4). The relative potencies of these compounds as antagonists at β_1-receptors is MDL 17,330A > medroxalol > MDL 17,331A, whereas the inverse potency relationship is observed at α_1-receptors. We studied the effects of these enantiomers and of medroxalol at α_1-, α_2- and β_2-receptors. The blockade of α_1-receptors was measured as antagonism of the pressor response to phenylephrine in anesthetized rats. Drug effects at α_2-receptors were assessed by measuring *in vitro* contractile responses to clonidine of the canine saphenous vein, a tissue where postsynaptic α_2-receptors predominate over α_1-receptors (8). Finally, the blockade of drug induced hypotension by propranolol pretreatment in the rat was used to measure the β_2- agonist activity of medroxalol and its enantiomers.

MATERIALS AND METHODS

Antagonism of Postsynaptic α_1-Adrenergic Receptors In Vivo

Male Wistar rats (Harlan, 200-450 gm) were anesthetized with urethane (1.4 g/kg i.p.). Both adrenal glands were excised and a carotid artery was cannulated for blood pressure recording using Statham P23D blood pressure transducers and a Grass Polygraph. Adrenalectomized rats were used in both these experiments and where propranolol was administered to block vascular beta receptors (see below) to ensure comparable data. The adrenal glands were removed because propranolol (used below) causes adrenal catecholamine release (16) resulting in a change of blood pressure which would make data analysis more difficult were non-adrenalectomized rats used. Both femoral veins were cannulated for separate administration of agonists or antagonists. Following completion of surgical procedures on the anesthetized, adrenalectomized (AADX) rats, an equilibration period of 50 min elapsed. A maximally effective dose of phenylephrine (100 μg/kg i.v.) was administered to obtain the peak diastolic blood pressure response (+72.0 ± 1.9 mm Hg, n = 41) to postsynaptic α_1-adrenoceptor stimulation. This peak diastolic response to phenylephrine did not differ between treatment groups based upon one-way analysis of variance. This response was followed by a 30 min re-equilibration period. Medroxalol or an enantiomer was administered at 1, 3 or 10 mg/kg i.v. Twenty min later, cumulative doses (18) of phenylephrine were administered as 0.1 ml/kg bolus injections each at the time of maximal hypertensive response to the previous dose. Data were expressed as a percent of the increase in diastolic blood pressure caused by the 100 μg/kg dose of phenylephrine administered at the beginning of the experiment. Data were analyzed using a four-parameter logistic equation (ALLFIT, 7) which provided estimates of maximum response, minimum response, ED50 (the dose of agonist that produces 50% of the maximal effect) and the slope of the dose-response line for each experiment. The ED50's for phenylephrine in the presence or absence of antagonists

were obtained after the curves were constrained to be parallel as required for analysis according to Arunlakshana and Schild (1). The ED50 doses were then used to calculate dose ratios between treatment groups for calculation of DR_{10} values (dose of antagonist required to increase the ED50 of agonist ten-fold) using Schild plots (1).

Antagonism of Postsynaptic α_2-Adrenergic Receptors In Vitro

Canine saphenous veins, known to possess postsynaptic α_2-adrenergic receptors (8), were dissected from mixed breed dogs (12-29 kg) that had been anesthetized with sodium pentobarbital 35 mg/kg i.v. Veins were cut transversely into 1 cm segments, mounted on stainless steel wire trapezes as modified from a previously described technique (12) and placed in a 50 ml organ bath containing warmed (37° C) oxygenated (95% O_2, 5% CO_2) Kreb's-Ringer bicarbonate solution of the following composition (mM): NaCl,119; KCl, 4.75; $CaCl_2$, 2.54; KH_2PO_4, 1.19; $MgSO_4$, 1.19; $NaHCO_3$, 25.0; D-glucose, 10.0; ascorbic acid, 2.8; and $\pm$ propranolol (10^{-6}M). One of the wire trapezes was secured to a muscle holder in the organ bath while the other was suspended from a Grass FT.03 force-displacement transducer. Isometric contractile force was recorded on a Grass Polygraph. Approximately 2 gms of tension were imposed upon the tissue which then equilibrated for 1 to 2 hours. Following equilibration, the tissue was exposed to a high K^+ (67 mM) Kreb's-Ringer solution (NaCl replaced in equimolar amounts by KCl) to produce a depolarization-induced contraction, followed by washout in normal Kreb's-Ringer solution and re-equilibration. Cumulative concentrations (18) of adrenergic agonists were added to the bath in the presence or absence of antagonists. Data were expressed as a percent of the maximal contractile response to KCl and analyzed using the computer program ALLFIT (7) as described above. Statistical significance between curves was ascertained using the analysis of variance tests in the ALLFIT program.

Effects of Propranolol on Blood Pressure Response to Medroxalol and Enantiomers

AADX rats were prepared as described above. After equilibration for 50 minutes, propranolol 1 mg/kg i.v. or saline was administered. This dose of propranolol was found sufficient to block vascular β_2-adrenergic receptors as judged by the complete antagonism of the cardiovascular sequelae of a maximally effective dose of isoproterenol (0.5 μg/kg i.v.) administered 15 min after propranolol. The effects of medroxalol, MDL 17,330A or MDL 17,331A (1-10 mg/kg i.v.) on diastolic blood pressure were tested in the absence or presence of propranolol (15 min pretreatment) and were analyzed for both absolute change and percent change of diastolic blood pressure for a 30 min period after administration of test compounds.

Data were compared using a univariate analysis of variance (3,14,19) on the maximal change in diastolic blood pressure at 5 min intervals over the 30 min post-treatment observation period. Also, a multivariate analysis of variance (3,13,14) was performed on the set of responses for each compound at each dose and time, and these parameters were subjected to contrast tests of compound and dose, i.e., each compound was contrasted to the other compounds, each dose of compound was contrasted to the other compounds at that dose, and each compound was contrasted for linear and quadratic dose-response relationships. Furthermore, the data for individual doses of each compound with and without propranolol pretreatment were compared using a two-way analysis of variance. In addition, a one-way analysis of variance was used to compare the hypotensive effects of medroxalol and its enantiomers using pooled data from all experiments in which the compounds were administered to AADX rats. In all statistical tests, a p-value of less than 0.05 was used to establish statistical significances.

Drugs

Phenylephrine hydrochloride, norepinephrine bitartrate, isoproterenol bitartrate and propranolol hydrochloride were purchased

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