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β-Adrenoceptor antagonists and human sperm motility

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Abstract—Several β-adrenoceptor blocking agents have been evaluated for spermicidal activity using a transmembrane migration method. The rank order of potency of the active compounds was: penbutolol > (+) - propranolol > bufuralol > (−) - alprenolol > oxprenolol > metoprolol. Atenolol, pindolol, practolol, tolamolol were without activity. The observed potencies of spermicidal activity are believed to be unrelated to β-blocking activities, and we have shown that whilst they are not predictable from lipid solubility or non-specific membrane properties of the compound alone, both these aspects appear to play a role in this pharmacological activity.

Many β-adrenoceptor antagonists, in addition to their specific cardiovascular therapeutic effect, exert a non-specific action on membranes which has been termed 'membrane stabilizing activity'. This property has not been clearly defined and encompasses a spectrum of non-specific membrane effects unrelated to the β-receptor antagonist activity, including local anaesthetic or quinidine-like effects, physical stabilization of membranes and protection against cell lysis (Smith 1982). These properties have been demonstrated with propranolol, a chiral member of the β-adrenoceptor antagonist family. Both optical isomers, (+)- and (−)-propranolol possess membrane stabilizing activity, whereas the (+)-isomer is only a weak β-adrenoceptor antagonist (Barrett & Cullum 1968). Peterson & Freund (1973) demonstrated that both the racemic mixture and (+)-propranolol inhibited human sperm motility at millimolar concentrations, compatible with "membrane stabilizing activity" involvement. We have thus investigated the effects on human sperm motility of several β-adrenoceptor antagonists, with and without previously reported non-specific membrane stabilizing activity and having a broad range of lipid solubilities.

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Methods

The modified transmembrane migration method of Hong et al (1981a), as previously described (Gadd & Curtis-Prior 1988), was used to measure the effects on sperm motility of the following β-adrenoceptor antagonists: (±), (+), and (−)-propranolol hydrochloride (Sigma and ICI); bufuralol hydrochloride (Roche); (−)-alprenolol (Sigma); penbutolol sulphate (Hoechst); oxprenolol hydrochloride (Ciba-Geigy); metoprolol tartrate (Ciba-Geigy); tolamolol hydrochloride (Pfizer); pindolol (Sandoz); practolol (ICI) and atenolol (ICI). All drugs were dissolved in 0·9% w/v NaCl saline or phosphate buffered saline (Dulbecco "A"). Dose-response curves were constructed on each ejaculate with triplicate measurements of motility at each concentration. The data were analysed using PCONLIN, an iterative non-linear regression analysis program to fit the parameters of the following equation:

$$I = I_0 - \frac{I_0 C^s}{Q^s + C^s}$$

where I_0 is the percentage inhibition of motility at zero concentration of inhibitor drug, C is the variable drug concentration, Q (IC50) is the drug concentration at which 50% maximal inhibition occurs, and S is a parameter controlling the "sigmoidicity" of the response curves.

Results and discussion

In agreement with previous observations (Peterson & Freund 1973; Hong et al 1981a), the racemate and (+)-/(−)-isomers of propranolol (0·1 to 10 mM) produced a dose-dependent inhibition of sperm motility, the concentrations producing 50% inhibition of motility (IC50) being 1·26±0·09, 1·3±0·11 and 1·65±0·21 mM, respectively (Fig. 1). The IC50 value obtained

for (+)-propranolol was close to the 1·3 mM obtained by Hong et al (1981a, b). Also, like Hong et al, we found no significant difference between the effect of the (+)-isomer and the racemate. Both isomers exhibit non-specific membrane effects, at concentrations several fold higher than is necessary for β-adrenoceptor antagonist activity, although the precise mechanism of the non-specific membrane activity is unknown. However, in erythrocyte membranes it has been shown that propranolol causes configurational changes in the membrane proteins, including the phospholipid components (Godin et al 1976), and that these changes include alterations in functionally significant membrane/cation interactions. It is not known if this occurs in the human spermatozoa membrane.

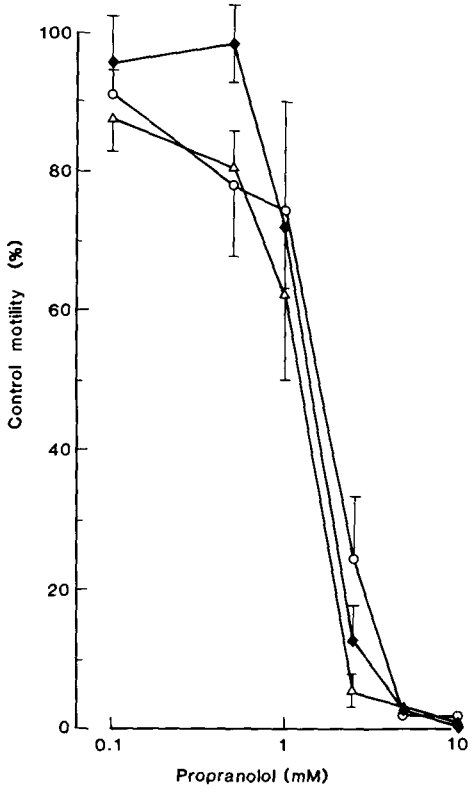

FIG. 1. Effects of propranolol ((+)-, ◆; (−)-, ○ and (±), △) on human sperm motility, expressed as a percentage of the control (nil drug) value. Each curve shows means from 4 or 5 sperm donors (mean ± s.e.m.)

Fluorescence microscopy using ethidium bromide indicated that (+)-propranolol has a spermicidal, rather than spermiostatic, mechanism of action; the dose-response curve for the percentage of dead spermatozoa produced by propranolol correlated with its sperm immobilizing activity (unpublished observations).

In addition to propranolol, other β-adrenoceptor antagonists also possess this non-specific membrane activity. However, there is controversy about whether this action is related to hydrophilicity. Previous investigations of myocardial and local anaesthetic actions of β-adrenoceptor antagonists (Levy 1968) showed no simple relation between these actions, whereas Hellenbrecht et al (1973) found a highly significant correlation. Moreover, Hong & Turner (1982) studied the action of β-adrenoceptors on sperm motility and found a linear correlation between sperm immobilizing activity and lipid solubility. However, no β-blocking drug with a higher lipid solubility than propranolol was studied. They also showed that sotalol, a β-adrenoceptor antagonist possessing no membrane stabilizing activity (Smith 1982), still inhibited sperm motility, but at high concentrations. Consequently, we have studied the effect of a variety of β-adrenoceptor antagonists possessing a wide range of partition coefficients, with and without non-specific membrane activity.

The effects of penbutolol, (+)-propranolol, (−)-alprenolol, oxprenolol and metoprolol (0·1–100 mM) are shown in Fig. 2. The dose-response curves for sperm motility produced by these β-adrenoceptor antagonists were parallel, with penbutolol being the most potent and metoprolol the least potent. Tolamolol, pindolol, practolol and atenolol (0·1–25 mM) had no effect on sperm motility.

Table 1 details the IC50 values of the sperm immobilizing potency of all the drugs studied, their respective octanol/aqueous buffer partition coefficients (Hinderling et al 1984) and a qualitative indication of their reported non-specific membrane activity (Smith 1982). There was a correlation between the inhibition of sperm motility by β-adrenoceptor antagonists and lipid solubility (correlation coefficient $r = 0.933$, see Fig. 3).

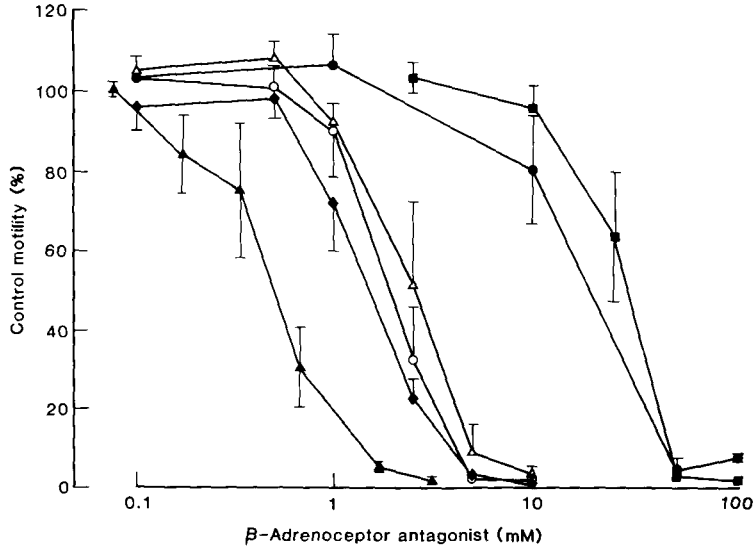

FIG. 2. The effects of increasing concentrations of various β-adrenoceptor blocking agents on human sperm motility, expressed as a percentage of the control (nil drug) value: penbutolol (▲), (+)-propranolol (◆), bufuralol (○), (−)-alprenolol (△), oxprenolol (●), and metoprolol (■).

Table 1. Sperm immobilizing potency, lipid solubility and non-specific membrane activity of β-adrenoreceptor antagonists.

β-Adrenoceptor antagonist	IC50 (mM)[a]	K′[b]	NSM[c]
Penbutolol	0·51 ± 0·05 (5)	81·0	+
(+)-Propranolol	1·26 ± 0·09 (5)	13·5	+
Bufuralol	1·92 ± 0·14 (3)	62·7	+
(−)-Alprenolol	2·38 ± 0·21 (4)	9·5	+
Oxprenolol	15·43 ± 3·61 (4)	1·6	+
Metoprolol	22·77 ± 1·47 (4)	0·5	−
Tolamolol	no effect	33·0	−
Pindolol	no effect	0·4	+
Practolol	no effect	0·05	−
Atenolol	no effect	0·02	−

(a) IC50: mean ± standard error of (n) experiments
(b) K′: apparent octanol:buffer partition coefficient at pH 7·4 determined by the shake flask method (data from Hinderling et al 1984).
(c) NSM: non-specific membrane activity (data from Smith 1982).

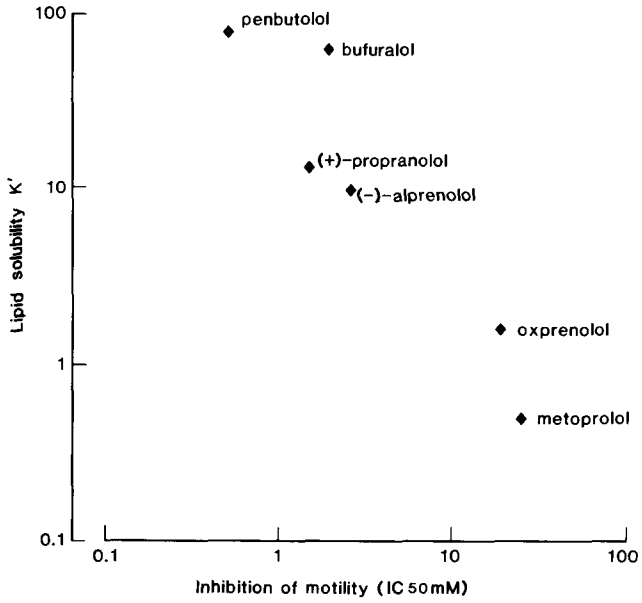

FIG. 3. Relationship between lipid solubility (expressed as K′, apparent octanol:aqueous buffer partition coefficient at pH 7·4) and the spermicidal activity of penbutolol, bufuralol, (+)-propranolol, (−)-alprenolol, oxprenolol and metoprolol.

However, our results suggest that the potency of a given drug in inhibiting sperm motility cannot be predicted from either its lipid solubility alone (tolamolal, which has a partition coefficient three fold greater than propranolol, had no effect on sperm motility) or previously-determined membrane stabilizing activity alone (metoprolol inhibited sperm motility but has no "membrane stabilizing activity", and vice versa for pindolol). However, high lipid solubility and membrane stabilizing activity are found in five of the six active compounds, so these two criteria would appear to play a role, still to be elucidated, in the spermicidal activity of β-adrenoceptor antagonists.

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