INFLUENCE OF LIPID SOLUBILITY ON THE SPERM IMMOBILIZING EFFECT OF β -ADRENOCEPTOR BLOCKING DRUGS

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- 1 Sperm immobilizing effects of five β -adrenoceptor blockers were measured on six human semen samples with a trans-membrane migration method.
- 2 The concentrations which decreased sperm motility to 50% of control (ED_{50}) were 0.8, 4.2, 6.2, 11.0 and 33.0 mM for propranolol, oxprenolol, metoprolol, acebutolol and sotalol respectively.
- 3 There was a linear correlation between the log values of ED_{50} and the log partition coefficients of these β -adrenoceptor blockers.
- 4 Because the lipid solubility determines the sperm immobilizing potencies of β -adrenoceptor blockers, we support the theory that β -adrenoceptor blockers inhibit sperm motility by stabilizing the cellular membrane.
- 5 The clinical implication of this finding is that more lipid soluble drugs are likely to have more interference on sperm function, at least when they are used locally.

Introduction

There is ample evidence that local anaesthetic drugs act on the cell membrane (Seeman, 1972) and the term 'membrane stabilizing effect' is frequently interchanged with 'local anaesthetic effect' in the literature, especially when referring to β -adrenoceptor blocking agents. Such an action of β -adrenoceptor blockers may not be associated with their clinical use as anti-hypertensive (Simpson, 1974) and antianginal drugs (McDevitt, 1977), although this action was considered to be responsible, at least in part, for the shift in the haemoglobin-oxygen dissociation curve to right *in vitro* (Trembath *et al.*, 1981) as well as to the decrease in conversion of l-thyroxine to l-tri-iodothyronine both *in vitro* (Heyma *et al.*, 1980a) and *in vivo* (Heyma *et al.*, 1980b). Propranolol can abolish the motility of washed suspension of human sperm (Peterson & Freund, 1975). It has also been shown to inhibit the motility of fresh human sperm in a concentration-response fashion (Hong *et al.*, 1981b). Because the dextro isomer of propranolol is as potent as its racemic isomer in this effect, membrane stabilization is the most likely underlying mechanism.

There is great variation among the potencies of β -adrenoceptor blockers as local anaesthetics and in their lipid solubility. Hellenbrecht *et al.* (1973)

showed that the local anaesthetic effect of β -adrenoceptor blockers was closely related to their octanol/buffer partition coefficients. There were also reports correlating the lipid solubility of β -adrenoceptor blockers with their binding to plasma protein, penetration to brain tissue and extent of metabolism in liver (Cruickshank, 1980).

Following the proposal that human sperm, being a spontaneously active cell, can be a potential model for studying local anaesthetics (Hong *et al.*, 1982), we have used different β -adrenoceptor blockers with various lipid solubilities to investigate their sperm immobilizing effects. By doing so, a better understanding of the interrelationship between the inhibition of sperm motility, local anaesthetic effect, membrane action and lipid solubility of β -adrenoceptor blockers might be provided.

Methods

Fresh human semen samples were collected from either healthy volunteers or patients attending the seminology laboratory at our hospital. Only samples with sperm concentrations higher than $15 \times 10^6 \text{ ml}^{-1}$

and percentage of progressive forward moving sperms higher than 20% were used because a previous study (Hong *et al.*, 1981a) had shown that motility measurement was less accurate in poor motility samples

All drugs were dissolved in phosphate buffered saline at pH 7.3 (Dulbecco 'A', Oxoid Ltd). Each semen sample was divided into several 100 μ l aliquots which were then mixed with 50 μ l of drug solution or phosphate buffer. The motility of sperm in semen-buffer mixture was used as control and those of semen-drug mixtures were expressed as percentage of control.

Determination of sperm motility was carried out as previously described (Hong *et al.*, 1981a). 100 μ l of semen-buffer or semen-drug mixture was pipetted into a small tube to the lower end of which a sheet of Nuclepore membrane with many evenly distributed 5 μ m pores had been bonded. This tube was then inserted into a glass bottle which contained phosphate buffer. The proportion of sperms that move across the membrane during 2 h incubation at 37°C was called trans-membrane migration ratio, which is a simple, objective and reproducible expression of sperm motility.

Drugs tested in this experiment include propranolol HCl (ICI), oxprenolol HCl (Ciba), metoprolol tartrate (Geigy), acebutolol HCl (M & B) and sotalol HCl (Mead Johnson). Six semen samples were used for each drug. The concentrations of drugs that decreased sperm motility to 50% of control (ED_{50}) were obtained from the semilogarithmic concentration-effect curves. The linear correlation between lipid solubility and sperm immobilizing effect was calculated by using the Standard Pac of a Hewlett Packard 85 computer.

Results

Figure 1 shows the dose-dependent lowering of the sperm motility by propranolol, oxprenolol, metoprolol and acebutolol. Sotalol can only partially

Figure 1 Log concentration-response curves for the inhibition of human sperm motility by β -adrenoceptor blockers. The motility of sperms in semen-buffer mixture was used as control. The concentrations of drugs are expressed in mM. All points are mean $\pm$ s.e. mean of six samples.

inhibit sperm motility at concentrations as high as 30 mM. All the dose-response curves are parallel.

Table 1 shows the concentration of β -adrenoceptor blockers required to decrease sperm motility to 50% of control (ED_{50}), the log value of each ED_{50} , partition coefficients n-octanol/buffer at pH 7.4 and 37°C, and the log values of partition coefficients.

Because sotalol could not inhibit sperm motility to 50% of control even at the highest concentration tested in this experiment, its ED_{50} was extrapolated from the dose-response curve available.

Woods & Robinson (1981) demonstrated that the partition coefficients of β -adrenoceptor blockers varied according to pH and temperature. We chose the value reported by them at pH 7.4 and 37°C because it was the closest to our experimental condition. The pH of drug solutions in our experiment was 7.3. We have studied the influence of pH on human sperm motility and found that human sperms had a fairly constant level of trans-membrane migration ratio over the pH range of 6.5 to 8.0 (unpublished data) so the difference between 7.3 and 7.4 was not considered to be significant.

Table 1 Sperm immobilizing potency and lipid solubility of β -adrenoceptor blockers

Drugs	ED_{50} * (mM)	Log ED_{50}	Partition † coefficients	Log partition coefficients
Propranolol	0.8	-0.09	20.2	1.30
Oxprenolol	4.2	0.62	2.28	0.35
Metoprolol	6.2	0.79	0.98	-0.008
Acebutolol	11.0	1.04	0.68	-0.16
Sotalol	33.0**	1.51	0.039	-1.40

* concentrations of drugs that decrease sperm motility to 50% of control.

** extrapolated from the dose-response curve.

† n-octanol/buffer, pH 7.4 and 37°C (Woods & Robinson, 1981).

The correlation between lipid solubility and sperm immobilizing effect of β -adrenoceptor-blockers is shown in Figure 20. Partition coefficients and ED_{50} plotted on the log scale were used as the parameters of lipid solubility and sperm immobilizing effect respectively. The correlation coefficient was 0.982 and the regression equation was $y = -1.62x + 1.27$.

Figure 2 Correlation between lipid solubility (partition coefficient) and sperm immobilizing potency (ED_{50}) of β -adrenoceptor blockers. Both partition coefficient and ED_{50} are plotted on log scale. $r = -0.982$.

Discussion

This experiment demonstrated that the sperm immobilizing effect of β -adrenoceptor blocking drugs is related to their lipid solubility. For more lipid-soluble β -adrenoceptor blockers, lower concentrations are required to inhibit human sperm motility. The relationship can be described by the equation:

$$\log P = -1.62 \log ED_{50} + 1.27$$

This equation is in the form of the Freundlich Isotherm (Barlow, 1980) which has been used to describe the adsorption of molecules on the surface of some solids. This equation is also similar to that described by Jack (1981) relating the protein binding of β -adrenoceptor blockers to partition coefficient. It is likely that the β -adrenoceptor blockers exert their immobilizing effect by being absorbed onto the sperm membrane.

The application of this mathematical relationship to predict the sperm immobilizing potency, a physiological property, from partition coefficient, a physicochemical property, or *vice versa*, gives further evidence that human sperm is a simple and useful pharmacological model.

Among different β -adrenoceptor blockers, propranolol has the most prominent local anaesthetic activity while sotalol has little or no such activity (Frishman, 1979). Peterson & Freund (1975) re-

ported that sotalol could inhibit sperm motility. Its potency, although lower than propranolol, was higher than lignocaine in their study. It has been shown that the ED_{50} for lignocaine to inhibit human sperm motility measured with our trans-membrane migration method is 16 mM (Hong, 1982). That sotalol can not inhibit sperm motility to 50% of control at 30 mM as shown in this experiment correlates better with its lack of potency as a local anaesthetic. The microscopic examination method used by Peterson & Freund (1973, 1975) was subjective and not accurate enough for research purposes; artifacts might also be introduced during their procedures to prepare a washed suspension of sperm from pooled human semen samples.

The local anaesthetic effects of metoprolol (Åblad *et al.*, 1975), oxprenolol (Vaughan Williams & Papp, 1970) and acebutolol (Basil *et al.*, 1973) have been reported to be less than that of propranolol. This is in agreement with their sperm immobilizing effects found in this experiment.

The technique for measuring trans-membrane potential with microelectrodes may be difficult to apply in motile sperms, and therefore, it is impossible at present to prove that β -adrenoceptor blockers modify sodium conductance of the sperm membrane in the same way that local anaesthetics influence nerve cell membrane. However, from the above discussion and the fact that lipid solubility determines the local anaesthetic activity of β -adrenoceptor blockers measured from frog sciatic nerve (Hellenbrecht *et al.*, 1973), it can be concluded that sperm immobilization is another manifestation of a local anaesthetic effect of β -adrenoceptor blockers, and the primary site of action is the cellular membrane, even though metabolic and enzymatic activities have been found to be changed after mixing sperm with membrane active drugs (Peterson & Freund, 1973; Tamblyn & First, 1977). In fact, it raises the question whether membrane bound enzymes or microfilaments connected to cell membrane are the regulators of sperm movement as in several other motile eukaryotic cells (Cappuccinelli, 1980).

The clinical implication of this experiment, other than a better understanding of sperm physiology and pharmacology, is that more affinity to the cell membrane, as well as more interference with sperm motility might be expected from more lipid soluble drugs, which in turn could be distributed to a greater extent in the tissues, including male reproductive organs (Okumura *et al.*, 1975). On the other hand, lipid soluble drugs are more subject to liver metabolism which may render them inactive. The overall relationship between lipid solubility and interference of drugs on sperm function *in vivo* needs further investigation, preferably based on the analysis of individual drug studies. As Mann & Lutwak-Mann (1981) concluded in their comprehensive review of

andrological pharmacology, in spite of its fundamental importance, this is still a largely unmapped area and thus a major, preferably multi-disciplinary effort is needed here. Even when it is argued that the concentrations of drugs in the genital tract are unlikely to be high enough for inhibiting sperm motility when they are administered systemically, the contribution of lipid solubility should still be considered when local application of these drugs as a contraceptive is proposed.

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