Local anaesthetic effect of antiarrhythmic drugs and human sperm immobilization: mechanism and application of the interrelationship

C. Y. HONG & B. N. CHIANG

Clinical Research Centre and Cardiology Section, Department of Medicine, Taiwan Veterans General Hospital and National Yang-Ming Medical College, Taipei, Taiwan, Republic of China

- 1 Sperm immobilizing activity of three class I antiarrhythmic drugs, quinidine, procainamide, mexiletine, one adrenoceptor blocking drug, labetalol and one calcium channel blocking drug, diltiazem was measured with a trans-membrane migration method.
- 2 All these drugs immobilized human sperm. Local anaesthetic effect is the underlying mechanism for these drugs to inhibit sperm motility.
- 3 Sperm immobilization could be a screening test for the local anaesthetic effect of antiarrhythmic drugs.

Keywords antiarrhythmic drugs contraceptive local anaesthetic effect propranolol sperm motility

Introduction

Antiarrhythmic drugs are classified by Vaughan-Williams (1981) according to their cellular electrophysiological properties. Compounds which have class I action on the cardiac membrane are also local anaesthetic on nerve. Local anaesthetic effect is thus considered as a characteristic pharmacological property of class I antiarrhythmic agents. However, this effect can be demonstrated in some drugs which form other electrophysiological classes. For example, propranolol, a β -adrenoceptor blocking agent with class II action, is known to possess local anaesthetic effect. Some calcium antagonists (class IV antiarrhythmic drugs), such as diltiazem and verapamil, were also found to have local anaesthetic effect (Nayler & Horowitz, 1983).

Human sperm is a potential model for pharmacological and toxicological research. Concentration-dependent inhibition of local anaesthetics on human sperm motility has been shown by Hong (1982a) with a trans-membrane migration method. By comparing the sperm

immobilizing potencies of various β -adrenoceptor blocking drugs, Hong & Turner (1982) considered that sperm immobilization is a manifestation of local anaesthetic effect. The involvement of mitochondrial respiratory enzymes in human sperm immobilization has recently been excluded (Hong *et al.*, 1983). All these findings support that human sperm could be an ideal *in vitro* model for screening the local anaesthetic activity of drugs.

The cellular electrophysiological properties may have no clinical value in the management of arrhythmia (Hillis & Whiting, 1983); and the *in vitro* sperm immobilizing activity may not be related to the adverse effect of these drugs on fertility, at least when these drugs were taken by oral or parenteral routes (Hong & Turner, 1982). However, screening the local anaesthetic activity provides a useful preclinical test for potential antiarrhythmic drugs (Kumana & Hamer, 1979) and the sperm immobilizing effect may have application in the development of

vaginal spermicides (Turner & Hong, 1982). In addition, the presence of local anaesthetic effect reflects to some extent the physicochemical properties of drugs, which in turn are related to the pharmacokinetic characters of drugs (Hong, 1982b). We therefore undertook a study to compare the sperm immobilizing potencies of antiarrhythmic drugs with a trans-membrane migration method.

The accuracy and precision of the trans-membrane migration method have been extensively studied (Hong *et al.*, 1981a; Hong, 1982b), but the application of this method is limited sometimes by the hydrophobic nature of the tested substances. In previous experiments (Hong *et al.*, 1981c, 1983), several kinds of solvents were used for dissolving water-insoluble drugs. These solvents were found to have little influence on the basal motility of human sperm, but whether solvents change the sperm immobilizing potency of drugs needs further investigation. A solvent containing 5% ethanol was used in this study to dissolve antiarrhythmic drugs with poor water solubility. A supplementary experiment which compares the potency of a drug in 5% ethanol and in phosphate buffered saline is included in this report.

Methods

Three class I antiarrhythmic drugs, namely quinidine sulphate (Sigma), procainamide HCl (Sigma), mexiletine HCl (Boehringer Ingelheim, FRG), one adrenoceptor blocking drug, labetalol (Glaxo, UK) and one calcium channel blocking drug, diltiazem (Tanabe, Japan) were tested in this study. Mexiletine was dissolved in phosphate buffered saline (pH 7.3, Dulbecco 'A'), quinidine, procainamide and labetalol were dissolved in a solvent containing 5% ethanol in phosphate buffered saline. Two test solutions of diltiazem were prepared, with phosphate buffered saline and 5% ethanol as solvent respectively.

Fresh human semen samples were collected from healthy donors. At least four samples were tested for each drug. All semen samples fulfilled previous criteria for trans-membrane migration studies (Hong *et al.*, 1981a). The trans-membrane migration method evaluated human sperm motility by measuring the proportion of sperm that moved across a Nuclepore membrane (Nuclepore Ltd USA) during 2 h incubation at 37°C. Details of this method were described elsewhere (Hong *et al.*, 1981a; Hong, 1982b). Motility of sperm in semen-solvent mixture was used as control and those in semen-drug mixtures were expressed as percentage of control.

The concentration of drug that decreased sperm motility to 50% of control (EC_{50}) was obtained from the concentration-response curve. A paired *t*-test was used to compare the difference between the potencies of diltiazem in phosphate buffered saline and in 5% ethanol. $P < 0.05$ was considered to be statistically significant.

Results

Figure 1 shows the concentration-dependent inhibition of five tested drugs on human sperm motility. The concentrations that decreased sperm motility to 50% of control (EC_{50}) were 0.5, 1.2, 1.4, 2.2, 2.8 and 20 mM for quinidine, diltiazem in 5% ethanol, diltiazem in phosphate buffered saline, labetalol, mexiletine and procainamide respectively. The differences between the sperm immobilizing potencies of diltiazem in 5% ethanol and in phosphate buffered saline were not statistically significant at any concentration (paired *t*-test, $P > 0.05$).

Discussion

There are three subtypes of class I antiarrhythmic drugs (Hills & Whiting, 1983). Mexiletine is Ib, while quinidine and procainamide are Ia drugs. Because flecainide, a Ic drug, also inhibits human sperm motility (Penhall *et al.*, 1982), sperm immobilizing effect is unlikely to be associated with any particular subtype of class I antiarrhythmic drug.

Adrenergic blocking drugs constitute class II antiarrhythmic drugs. Some of them are known to have local anaesthetic effects. Hong & Turner (1982) compared the sperm immobilizing activity of several β -adrenoceptor blocking agents, including propranolol, metoprolol, oxprenolol and acebutolol. β -adrenoceptor control of sperm motility was excluded by Hong *et al.* (1981b) who showed that isoprenaline did not modify sperm motility. In the present study, the EC_{50} of labetalol, a drug which blocks both α - and β -adrenergic receptors, was 2.2 mM. Indoramin, a selective α_1 -adrenoceptor antagonist, was found by Verma *et al.* (1983) to inhibit human sperm motility with an EC_{50} of 4.0 mM. The association between α -adrenoceptor and human sperm motility can almost certainly be excluded because noradrenaline can neither stimulate nor inhibit human sperm motility. The EC_{50} of clonidine, a selective α_2 -adrenoceptor agonist, was 11.0 mM (Hong, unpublished observations). Sotalol, a drug with both class II and class III action, was found to have little sperm immobilizing effect. This is compatible with its

Figure 1 Concentration-dependent inhibition of human sperm motility by quinidine (●), diltiazem (○), labetalol (▼), mexiletine (▲) and procainamide (■). Solid lines indicate drugs dissolved in 5% ethanol; broken lines indicate drugs dissolved in phosphate buffered saline. Sperm motility was measured with a trans-membrane migration method. Motility of sperm in semen-solvent mixtures were used as control, those in semen-drug mixtures were expressed as percentage of control. All points are mean $\pm$ s.e. mean for at least four semen samples.

lack of local anaesthetic activity (Hong & Turner, 1982).

Some calcium antagonists (class IV antiarrhythmic drugs), such as diltiazem and verapamil, were found to have local anaesthetic effect (Nayler & Horowitz, 1983). In this experiment, we demonstrated that diltiazem had potent sperm immobilizing effect which indicated the presence of local anaesthetic activity.

Although 5% ethanol tended to decrease the EC_{50} of diltiazem, the difference between the potencies of diltiazem in 5% ethanol and in phosphate buffered saline was not statistically significant. It is therefore acceptable to compare data derived from experiments using either 5% ethanol or phosphate buffered saline as solvents.

Local anaesthetic activity has in the past been used synonymously with membrane stabilizing activity or quinidine-like activity, especially in regard to β -adrenoceptor blocking drugs. These terms have been applied to several non-receptor mediated pharmacological actions on membranes, such as inhibition of myocardial lysosome lysis, protection against erythrocyte lysis or inhibition of phospholipase activity (Smith, 1982). It is apparent that there is a need to redefine and separate these properties. Only inhibition of sodium fast channel or slowing of neural conduction should be considered as local anaesthetic in nature, other membrane activity should be defined according to the particular type of action observed.

While sperm immobilization is a well defined pharmacological property and the presence of local anaesthetic activity has been demonstrated in conventional animal models for quinidine,

procainamide, mexiletine (Kumana & Hamer, 1979), labetalol (Farmer *et al.*, 1972) and diltiazem (Nayler & Horowitz, 1983), direct electrophysiological evidence which prove sperm immobilizing effect is identical to local anaesthetic effect cannot be provided because techniques using microelectrodes and voltage clamp may be difficult to apply to motile sperm. Recently, Garlid & Nakashima (1983) used a NH_4SCN swelling assay to study the mechanism of local anaesthetics on ionic transport. The relative potencies of drugs with local anaesthetic property in that assay followed the order: chlorpromazine > quinidine > propranolol > tetracaine > lignocaine > procaine > procainamide. The EC_{50} for the sperm immobilizing potencies of these drugs measured by the trans-membrane migration method were 0.2, 0.5, 0.8, 1.0, 16.0, 18.0 and 20.0 mM (Hong, 1982a; Hong *et al.*, 1981b, 1982). We consider that the theory proposed by Garlid & Nakashima (1983) which involved trans-membrane transport of ion pair could be responsible for the mechanism of sperm immobilization.

It is possible that local application of drug with local anaesthetic effect into the vagina before coitus would act as an effective contraceptive (Turner & Hong, 1982). A clinical study (Zipper *et al.*, 1983) involving a total of 127 women years shows a favourable result for the use of propranolol as a vaginal contraceptive. In addition to data presented in this study, more than 20 frequently prescribed drugs of various pharmacological categories but sharing a common local anaesthetic activity have been screened with the trans-membrane migration method. Propranolol, with its EC_{50} of 0.8 mM (Hong *et al.*, 1981b), is more potent than most other drugs in inhibiting human sperm motility. Although the sperm immobilizing potencies of quinidine and some psychotropic drugs (Hong, *et al.*, 1984) are higher than that of propranolol, the complicated systemic effects of the absorbed psychotropic drugs as well as the difficulty to prepare ideal dosage form from quinidine because of its poor water solubility, make propranolol, especially its dextroisomer which lacks cardiovascular activity but possesses potent local anaesthetic and sperm immobilizing effects (Hong *et al.*, 1981b), the drug of choice when this group of drugs is considered for vaginal contraception. The tolerability of the vagina to this form of treatment, however, remains to be determined (Patel *et al.*, 1983).

The authors thank Mr J. Ku and Ms P. Wu for technical assistance, Ms H. Y. Sun and Ms H. E. Tai for secretarial assistance. This study was supported by grants from the Department of Medical Research, Veterans General Hospital; Clinical Research Centre, Institute of Biomedical Sciences, Academia Sinica and National Science Council, Republic of China.

References

- Farmer, J. B., Kennedy, I., Levy, G. P. & Marshall, R. J. (1972). Pharmacology of AH5158: a drug which blocks both α - and β -adrenoceptors. *Br. J. Pharmac.*, **45**, 660–675.
- Garlid, K. D. & Nakashima, R. A. (1983). Studies on the mechanism of uncoupling by amine local anaesthetics. *J. biol. Chem.*, **258**, 7974–7980.
- Hills, W. S. & Whiting, B. (1983). Antiarrhythmic drugs. *Br. med. J.*, **286**, 1332–1336.
- Hong, C. Y. (1982a). Local anaesthetic agents immobilize human sperm. *J. Physiol. (London)*, **325**, 37P.
- Hong, C. Y. (1982b). *Measurement of human sperm motility in vitro, and the influence of some drugs upon it*. Ph.D. thesis, University of London.
- Hong, C. Y., Chaput de Saintonge, D. M. & Turner, P. (1981a). A simple method to measure drug effects on human sperm motility. *Br. J. clin. Pharmac.*, **11**, 385–387.
- Hong, C. Y., Chaput de Saintonge, D. M. & Turner, P. (1981b). The inhibitory action of procaine, d-propranolol and dl-propranolol on human sperm motility: antagonism by caffeine. *Br. J. clin. Pharmac.*, **12**, 751–753.
- Hong, C. Y., Chaput de Saintonge, D. M. & Turner, P. (1981c). Delta-9-tetrahydrocannabinol inhibits human sperm motility. *J. Pharm. Pharmac.*, **33**, 746–747.
- Hong, C. Y., Chaput de Saintonge, D. M. & Turner, P. (1982). Effects of chlorpromazine and other drugs acting on the central nervous system on human sperm motility. *Eur. J. clin. Pharmac.*, **22**, 413–416.
- Hong, C. Y., Chiang, B. N. & Ku, J. (1984). Sperm immobilizing potency of amitriptyline and imipramine: measured with trans-membrane migration. *Arch. Androl.* (in press).
- Hong, C. Y., Chiang, B. N. & Wei, Y. H. (1983). Mitochondrial respiration inhibitors and human sperm motility: implication in the development of spermicides. *Br. J. clin. Pharmac.*, **16**, 487–490.
- Hong, C. Y. & Turner, P. (1982). Influence of lipid solubility on the sperm immobilizing effect of beta-adrenoceptor blocking drugs. *Br. J. clin. Pharmac.*, **14**, 269–272.
- Kumana, C. & Hamer, J. (1979). Antiarrhythmic drugs. In *Drugs for Heart Disease*, ed. Hamer, J., p. 67. London: Chapman & Hall.
- Nayler, W. G. & Horowitz, J. D. (1983). Calcium antagonists: a new class of drugs. *Pharmac. Ther.*, **20**, 203–262.
- Patel, L. G., Warrington, S. J. & Pearson, R. M. (1983). Propranolol concentrations in plasma after insertion into the vagina. *Br. med. J.*, **287**, 1247–1248.
- Penhall, R. K., Hong, C. Y. & Muhiddin, K. A. (1982). The effect of flecainide on human sperm motility. *Br. J. clin. Pharmac.*, **14**, 147P.
- Smith, H. J. (1982). The need to redefine membrane stabilizing activity of beta-adrenergic receptor antagonists. *J. mol. cell. Cardiol.*, **14**, 495–500.
- Turner, P. & Hong, C. Y. (1982). The effects of propranolol and some other beta-adrenoceptor blocking drugs on human sperm motility. In *Progress towards a male contraceptive*, eds. Jeffcoate, S. L. & Sandler, M., pp. 221–225. Chichester: John Wiley.
- Vaughan Williams, E. M. (1981). Classification of antidysrhythmic drugs. In *Pharmacology of antiarrhythmic agents*, ed. Szekeres, L., pp. 125–150. Oxford: Pergamon.
- Verma, R. B., Abrams, S. M. L. & Turner, P. (1983). Effect of indoramin on human sperm motility. *Br. J. clin. Pharmac.*, **15**, 127–128.
- Zipper, J., Wheeler, R. G., Potts, D. M. & Rivera, M. (1983). Propranolol as a novel, effective spermicide: preliminary findings. *Br. med. J.*, **287**, 1245–1246.

(Received November 7, 1983,
accepted February 3, 1984)