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THE INHIBITORY ACTION OF PROCAINE, (+)-PROPRANOLOL AND (±)-PROPRANOLOL ON HUMAN SPERM MOTILITY: ANTAGONISM BY CAFFEINE

Caffeine and propranolol have been demonstrated respectively as a stimulator (Amelar, Dubin & Schoenfeld, 1980) and an inhibitor (Peterson & Freund, 1973; De Turner *et al.*, 1978) of human sperm motility. Further application of these findings awaits clarification of their mechanisms. While the stimulatory effect of caffeine may be a result of increased intracellular cAMP (Hoskins & Casillas, 1975), the inhibitory effect of propranolol is due to a drug-membrane interaction because its dextro isomer is as potent as the racemic isomer in this effect (Peterson & Freund, 1975). We have found that caffeine can antagonize the inhibitory effects of three known membrane stabilizers, procaine, (+)-propranolol and (±)-propranolol, and suggest that it owes its stimulatory property to a membrane action.

Fresh human semen samples were collected from either healthy volunteers or patients attending the seminology laboratory at our hospital. Only samples with a sperm concentration higher than $15 \times 10^6 \text{ ml}^{-1}$ and percentage of progressive forward moving sperms higher than 20% were used. All drugs were dissolved in phosphate buffered saline at pH 7.3 (Dulbecco 'A', Oxoid Ltd). Sperm motility was measured using the ability of forward moving sperms to move across the $5 \mu\text{m}$ pores of a Nuclepore membrane during 2 h incubation at 37°C (Hong, Chaput de Saintonge & Turner, 1981). The motility

of semen-buffer mixture was used as control and those of semen-drug mixtures were expressed as percentage of the control. Aliquots of semen were mixed with either buffer or drug in the ratio of 2 to 1. When two drugs were studied together, the volume of each drug was halved so that the ratio could be kept constant. The final concentration of caffeine was kept at 5 mM which was optimal for stimulating sperm motility (Hong *et al.*, 1981).

Six samples were tested for each drug. The concentrations which decrease the sperm motility to 50% of control were 18 mM for procaine, 1.3 mM for (+)-propranolol and 0.8 mM for (±)-propranolol. The difference between (+)-propranolol and (±)-propranolol was not statistically significant (Mann-Whitney U test, $P > 0.05$).

Figure 1a shows the log concentration-response curve for the antagonism between caffeine and procaine-inhibited sperm motility. Figure 1b and c show a similar effect of caffeine with (+)-propranolol and (±)-propranolol.

To exclude a chemical inactivation of propranolol by caffeine prior to its inhibition of sperms, we studied the sequence of mixing drugs and semen. It made no difference whether caffeine was administered before, concomitant with or 5 and 10 min after propranolol. Combined with the microscopic observation that propranolol inhibits sperm motility

Figure 1 Log concentration-response curves for the antagonism between caffeine and the inhibitory action of (a) procaine, (b) (+)-propranolol and (c) (±)-propranolol on human sperm motility. The motility was measured using the trans-membrane migration method (Hong *et al.*, 1981). The motility of semen-buffer mixture was used as control and those of semen-drug mixtures were expressed as percentage of the control. Semen was mixed with membrane stabilizers alone (●) or membrane stabilizers and 5 mM of caffeine (■). All points are mean $\pm$ s.e. mean of six samples. (*) indicates $P < 0.05$ (Sign test).

within seconds after mixing, we conclude that caffeine can antagonize propranolol at least 10 min after propranolol has immobilized sperms.

We could neither demonstrate the stimulatory effect of isoprenaline (10^{-2} to 10^{-8} M) nor reverse the propranolol-inhibited sperm motility with isoprenaline.

Although caffeine is a phosphodiesterase inhibitor, some of its pharmacological activities cannot easily be explained on this basis (Bolton, 1979). It has been shown that caffeine can act on the cellular membrane and is capable of direct activation of coupling mechanisms (Huddart & Syson, 1975; Thorpe & Seeman, 1971). Our findings suggest that a membrane action is at least in part responsible for the stimulation by caffeine of sperm motility. The extension of these *in vitro* findings to human fertility control awaits their *in vivo* validation as well as an assessment of their potential risk (Ronen & Marcus, 1978). Harrison, Sheppard & Kaliszer (1980) claimed that caffeine disrupts sperm architecture, even though it may enhance motility; an increase in motility may not necessarily be associated with increased fertility.

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SPURIOUSLY HIGH VALUES OF UNBOUND DRUG FRACTION IN SERUM AS DETERMINED BY THE NEW ULTRAFREE ANTICONVULSANT DRUG FILTERS

A new device (Ultrafree Anticonvulsant Drug Filters, Worthington Diagnostics) for the isolation and collection of non protein-bound anticonvulsant drug fractions for the analysis of free drug levels has recently been made commercially available. The technique involved is very simple and is based on the use of a filtering membrane, which is said to have a 40,000 molecular weight cut-off and to retain more than 99% of the plasma albumin (Worthington Diagnostics, 1979). Filtrate is collected by placing 2.0 ml of plasma into a disposable membrane cup fitted to a 1 ml tuberculin syringe. When the syringe plunger is withdrawn to provide a vacuum source, fluid is filtered through the membrane and is collected in the syringe. The concentration of the drug in a 0.2 ml protein-free filtrate should equal the concentration of unbound drug in the plasma. In the package insert (Worthington Diagnostics, 1979), users of the device are advised to analyse plasma rather than serum samples because 'results from experimental work were obtained with the exclusive use of plasma samples'. For many of the currently measured drugs, however, the unbound fraction in human plasma is known to be identical to that in serum (Weigand *et al.*, 1980). As many investigators find serum a more convenient fluid to use, we considered it of interest to evaluate the performance of the Filters with serum rather than with plasma, and to compare it with that of an established reference technique. In this letter, we provide evidence that the measurement of serum unbound drug concentration with the Ultrafree Drug Filters can lead to seriously erroneous results due to leakage of protein into the filtrate. These data are in partial agreement with those recently reported by other investigators (Moss, 1980; Joern, 1981).

Experiments were performed with pooled serum or plasma collected from epileptic patients receiving chronic therapy with phenytoin, usually in combina-

tion with other antiepileptic drugs. Samples (2.0 ml) were analysed fresh or after storage at -20°C for less than 3 months. Prior to separation of the unbound drug, a small amount (approximately $0.7\text{ }\mu\text{mol/l}$ serum or plasma) of [^{14}C]-labelled phenytoin (NEN) was dissolved in each sample. Experiments with the Ultrafree Drug Filters (kindly supplied by Worthington Diagnostics, Freehold, N.J.) were performed at room temperature ($22\text{--}23^{\circ}\text{C}$) and at 37°C according to the procedure described by the manufacturer. Care was taken to collect no more than 0.2 ml filtrate. Collection times ranged from 30 to 120 min at room temperature and from 15 to 70 min at 37°C . Experiments were also carried out by a reference ultrafiltration technique at room temperature according to the procedure described by Lunde *et al.* (1970) using Visking tubing (Scientific Supplies) and a MSE Super Minor Centrifuge. The unbound fraction of phenytoin was calculated as the ratio between the dpm in the (ultra) filtrate and the dpm in an equal volume of serum (plasma) prior to (ultra) filtration. The concentration of total (free + bound) phenytoin in the samples was determined by a modification of the gas-liquid chromatographic method described by MacGee (1970). The concentration of albumin in the (ultra) filtrate was determined according to Doumas, Watson & Briggs (1971) with minor modifications. The sensitivity of the method was 0.5 g/l .

Results of the reproducibility studies in (thawed) serum are summarised in Table 1. The unbound fraction of phenytoin as determined by the reference method was found to be 8.8% and 9.7% respectively in the two pools of serum examined. No albumin was detected in the ultrafiltrates. The within-assay variability of the method was below 3%.

Values of serum unbound phenytoin fraction as determined by using the Ultrafree Drug Filters Batch 1 (Lot unspecified) at room temperature were slightly