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Circadian changes in procainamide and *N*-acetylprocainamide kinetics in the rat

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The aim of this study was to investigate the possible influence of the time of administration on procainamide and *N*-acetylprocainamide (NAPA) kinetics in the rat. A single 50 mg kg^{-1} i.p. dose of procainamide was given to Wistar AF SPF adult male rats maintained under controlled environmental conditions (LD: 06.00h–18.00h) at four different fixed times i.e. 10.00, 16.00, 22.00 and 04.00h. Procainamide and NAPA plasma levels were determined by an immunoenzymatic method. Our data showed significant 24 h variation of the following pharmacokinetic parameters: highest elimination half-lives at 10.00h ($t_{\frac{1}{2}}\beta$ = 0·736 ± 0.020h) for procainamide and at 04.00h ($t_{\frac{1}{2}}\beta$ = 3·55 ± 0·08h) for NAPA ($P < 0·001$); highest apparent volume of distribution at 04.00h for procainamide (Vd = 2·35 ± 0·17 litre) ($P < 0·05$); highest ratio AUC NAPA/AUC procainamide at 04.00h (1·039 ± 0·056) ($P < 0·001$). Procainamide clearance and C_{max} and AUC for procainamide and NAPA were not significantly dependent on time of day. These data indicate a 24 h variation in the metabolism of procainamide which is converted to NAPA, the *N*-acetylation being greatest at 04.00h.

The existence of a pattern of drug effectiveness or kinetics is well known and has been demonstrated for several drugs (Reinberg & Halberg 1971). But little attention has been given to antiarrhythmic drugs from a chronopharmacological point of view. We have reported data on the chronopharmacokinetics of lignocaine in the rat (Bruguerolle et al 1982) and of disopyramide (Bruguerolle 1984).

This report examines the pharmacokinetic changes of procainamide and its major metabolite, *N*-acetylprocainamide (NAPA), related to the time of administration by assessing the rhythms in the pharmacokinetic parameters of this drug and metabolite after a single intraperitoneal dose in the rat.

Materials and methods

Wistar AF SPF adult male rats (n = 240) (~250 g) were housed, six to a cage, for a minimum of three weeks before use and with free access to food and water. Environmental conditions were controlled at relative humidity 50–55%, temperature 24 ± 2 °C, synchronization by natural light, 06.00–18.00h and darkness 18.00–06.00h, during the month of October. At 10.00, 16.00, 22.00 and 04.00h, a total of 240 animals, 60 per chosen time, were given procainamide chlorhydrate solution (25 mg ml^{-1}) intraperitoneally as a single 50 mg kg^{-1} dose; blood samples were collected by cardiac puncture 10, 15, 30 min and 1, 1·5, 2, 3, 4, 6 and 8 h after drug administration. Total procainamide and NAPA plasma levels were determined by an immunoenzymatic method (Emit, Syva-Biomerieux).

Procainamide and NAPA plasma concentrations were plotted against time and pharmacokinetic parameters were determined assuming a two or one compartment open model, respectively. Maximal concentration (C_{max}), elimination half-life ($t_{\frac{1}{2}}\beta$), clearance (Cl), apparent volume of distribution (Vd) and area under the plasma concentration curve (experimental and extrapolated to infinity) were assessed according to conventional methods (Gomeni & Gomeni 1978) by a computer program. The rate of biotransformation of procainamide to NAPA was expressed by the area under the NAPA concentration curve divided by the area under the procainamide concentration curve (AUC NAPA/AUC procainamide); K_{app} is the rate constant of metabolite appearance in the plasma.

All data were quantified (mean); differences were compared by conventional statistical analysis (analysis of variance, ANOVA).

Results

Table 1 shows the different pharmacokinetic parameters (mean) of procainamide after a single dose at 16.00, 22.00, 04.00 and 10.00h and statistical analysis (ANOVA) of the comparison. These results indicate a significant change in $t_{\frac{1}{2}}\beta$ being lowest at 16.00h and 22.00h, and in Vd (highest at 04.00h). C_{max}, AUC and clearance were not significantly different.

Table 2 shows the pharmacokinetic changes of NAPA with time. The rate constant of the metabolite appearance (K_{app}) in the plasma exhibits a rhythm, indicating that NAPA appears most rapidly when the drug is given at 04.00h.

Table 3 shows the change with time of the ratio AUC NAPA/AUC procainamide which indicates the intensity and amount of biotransformaton of procainamide to NAPA.

Discussion

Our data agree with the results of Kamath et al (1981) and demonstrate that the pharmacokinetics of procainamide and NAPA depend on the time of administration of procainamide. It is known (Reinberg & Smolensky

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Table 1. Procainamide pharmacokinetic parameters related to the hour of administration.

Time	C_{max}	$t_{\frac{1}{2}}\beta$	Vd	AUC exp.	AUC_0^∞	Cl
04.00	20·5 ± 0·9	0·728 ± 0·025	2·350 ± 0·170	18·89 ± 1·74	23·05 ± 2·05	2·275 ± 0·240
10·00	20·4 ± 1·7	0·736 ± 0·020	2·095 ± 0·138	21·84 ± 2·109	26·33 ± 2·51	2·000 ± 0·183
16·00	22·2 ± 1·54	0·658 ± 0·026	1·798 ± 0·124	22·79 ± 2·36	27·44 ± 2·74	1·931 ± 0·188
22·00	20·6 ± 1·58	0·637 ± 0·011	1·840 ± 0·118	21·42 ± 1·80	25·77 ± 2·13	2·015 ± 0·156
ANOVA	ns	$P < 0·001$	$P < 0·05$	ns	ns	ns

C_{max} (μg ml^{-1}) = maximal concentration. $t_{\frac{1}{2}}\beta$(h) = elimination half-life. Vd (litre kg^{-1}) = apparent volume of distribution. AUC exp. and AUC_0^∞ (μg ml^{-1} h) = area under the plasma concentration curves (experimental and extrapolated to infinity). Cl (litre h^{-1} kg^{-1}) = clearance. K_{app} = rate constant of metabolite appearance.

1982; Bruguerolle 1983) that chronokinetic differences can occur at one or more stages i.e. absorption, distribution, metabolism and excretion of the drug.

When procainamide is administered by the intraperitoneal route, the data do not reveal changes in its absorption with time of day (see C_{max} values in Table 1). Circadian changes in the apparent volume of distribution of the drug (Table 1) may result from circadian variations of blood volume and/or tissue perfusion or of protein binding of the drug but as procainamide is only 15% bound to plama proteins, a circadian change in protein binding would not affect the kinetic changes observed and so was not investigated.

Blood flow and blood volume are known to be greater during nocturnal activity than during diurnal rest in rodents (Lew 1976). Recently, Doré et al (1984) reported circadian variations of hepatic, renal, intestinal and muscular blood flow in rodents, with maximal values at 21.00 and 03.00h. The higher apparent volume of distribution we observed at 04.00h is therefore not surprising. Moreover, since rats feed primarily in the evenings, their eating habits could influence blood flow and so may be involved, to some extent, in the circadian variations of procainamide volume of distribution.

Schneck et al (1978) indicated that 34% of a 50 mg kg^{-1} dose of procainamide was excreted unchanged in the urine of rats, about 40% was converted to NAPA (*N*-acetylation being the major pathway of procainamide metabolism), the rest was eliminated as unidentified metabolites.

Kamath et al (1981) reported that in rodents the elimination half-life of procainamide was 0·658 ± 0·107 h, the elimination half-life of NAPA was 2·13 ± 0·36 h and the clearance of procainamide was 1·92 ± 0·42 litre. They also found the NAPA half-life to be approximately 3 times longer than that of procainamide. The circadian variations of procainamide and NAPA elimination half-lives found, agree with the results of Kamath et al (1981) when procainamide was administered at 10.00 or 16.00h; but when procainamide was given at 2.00 or 04.00h (during nocturnal activity) NAPA elimination half-life averaged as much as 4 to 5 times longer than of the parent compound. The rate constant of metabolite appearance (K_{app}, Table 2) is highest at 04.00h so that the ratio AUC NAPA/AUC procainamide: *N*-acetylation seems to vary with time of day with maximal values at 04.00h.

Table 2. NAPA pharmacokinetic parameters related to the hour of administration.

Time	C_{max}	K_{app}	$t_{\frac{1}{2}}\beta$	AUC exp.	AUC_0^∞
04.00	3.55±0·27	2·85±0·51	3·55±0·08	15·86±1·01	23·51±1·56
10·00	3·87±0·25	1·97±0·15	2·66±0·06	18·17±0·89	22·05±1·22
16·00	4·25±0·34	1·18±0·16	2·38±0·05	17·74±1·00	20·51±1·11
22·00	4·08±0·04	1·12±0·11	2·44±0·07	15·40±0·76	18·62±0·88
ANOVA	ns	$P < 0·001$	$P < 0·001$	ns	ns

Table 3. Circadian variation of the ratio AUC NAPA/AUC procainamide.

Time	AUC NAPA/AUC PA
04.00	1·0390 ± 0·0560
10.00	0·8584 ± 0·0406
16.00	0·7692 ± 0·0405
22.00	0·7360 ± 0·0304
ANOVA	$P < 0·001$

Temporal variations of drug metabolism have been reported for many drugs in experimental or clinical studies (Bruguerolle 1983, Reinberg & Halberg 1971, Reinberg & Smolensky 1982, Smolensky et al 1976) to depend on circadian changes in both hepatic blood flow or liver enzyme activity. Since procainamide is not avidly taken up by the liver (Koch-Weser 1975) its clearance does not depend on hepatic blood flow. Circadian variation in enzyme activity has been studied in-vitro: daily variations in the in-vitro metabolism of drugs by rat liver (oxidation, reduction, hydroxylation, demethylation) have been reported (Bruguerolle 1983; Reinberg & Smolensky 1982) but to our knowledge, circadian variations in acetylation have not been demonstrated.

Procainamide is used for the control of ventricular arrhythmias and NAPA, its major metabolite, is pharmacologically active (Elson et al 1975; Drayer et al 1974). Since there is a good relation between the antiarrhythmic activity and serum levels of procainamide and NAPA, the pharmacokinetic changes that we found may explain the circadian changes of procainamide and NAPA effectiveness and/or toxicity.

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Comparison of the affinity constant of some muscarinic receptor antagonists with their displacement of [^{3}H]quinuclidinyl benzilate binding in atrial and ileal longitudinal muscle of the guinea-pig

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The ability of the muscarinic receptor antagonists fenipramide, 4-diphenylacetoxy-*N*-methyl piperidine methiodide (4-DAMP) and secoverine to displace [^{3}H]QNB binding was correlated with the inhibition of responses of cholinomimetics at muscarinic receptors in the atria and ileal longitudinal muscle of the guinea-pig. Fenipramide and 4-DAMP exhibited a 2–4 fold higher affinity for muscarinic receptors in ileal longitudinal muscle in both types of experiments. Secoverine exhibited no difference in affinity in the two tissues.

Claims of heterogeneity amongst muscarinic receptors have been based partly on receptor binding studies using radiolabelled muscarinic receptor antagonists such as [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) (Ellis & Hoss 1982; Dunlap & Brown 1983) or [^{3}H]*N*-methylscopolamine (Hammer et al 1980; Stockton et al 1983) and partly on studies in-vivo or in-vitro measuring the relative potency or affinity of muscarinic receptor antagonists (Brown et al 1980; Hammer & Giachetti 1982). To determine whether there was a correlation in the two types of experiments, a comparison was made of the affinities of some muscarinic receptor antagonists determined in isolated tissue experiments in guinea-pig atria and ileal longitudinal muscle with the ability of these antagonists to displace [^{3}H]QNB binding in the tissues.

The muscarinic receptor antagonists investigated were 4-diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP), secoverine, fenipramide (HO 9980, α,α-diphenyl-γ-piperidyl butyramide HCl) and atropine. 4-DAMP has been reported to possess a higher affinity for muscarinic receptors in ileum than in atria (Barlow et al 1976, 1980). Secoverine also possesses selectivity for some muscarinic receptors as it blocks the receptors in ileum in lower doses than those required in salivary or lachrymal glands (Zwagemakers & Claassen 1980, 1981). Fenipramide was originally investigated by Schaumann & Lindner (1951) and shown to possess potent muscarinic receptor blocking activity with minimal antispasmodic activity. Its structure is related to that of difenidol, a compound which exhibits some selectivity for ileal muscarinic receptors over those in atria (Mutschler & Lambrecht 1984).

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Methods and materials

Concentration-response curves for the negative inotropic, negative chronotropic or contractile responses to carbachol or arecaidine propargyl ester were obtained in guinea-pig left atrium (driven at 3 Hz), right atrium (spontaneously beating) or ileal longitudinal muscle respectively. Tissues were bathed in McEwen's solution (McEwen 1956) at 37 °C and gassed with 95% oxygen and 5% carbon dioxide. Responses in duplicate were recorded isometrically under a resting tension of 0·5 g. Dose-ratios were calculated from the concentration giving 50% of the maximal responses before and after 40 min (fenipramide, secoverine) or 60 min (atropine, 4-DAMP) incubation with the antagonists. Mean pK_B values were estimated from Arunlakshana-Schild (A–S) plots when several concentrations of antagonist were employed. When only one concentration of antagonist [B] was used, a mean 'pA_2' value was estimated from the dose-ratio (DR) obtained, using the relation:

$$pA_2 = \log (DR - 1) - \log [B]$$