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Similar central actions of intravenous methohexitone suspension and solution in the rabbit

H. VIERNSTEIN, C. STUMPF, *Institute of Pharmaceutical Technology and Institute of Neuropharmacology, University of Vienna, Vienna, Austria*

Abstract—The central actions of solutions of methohexitone sodium and suspensions of methohexitone given intravenously in equimolar doses have been compared. The administrations induced identical anaesthesia. Because, in general, drugs acting upon the central nervous system are poorly soluble in water, this finding should be useful for routine pharmacological investigations as it avoids the use of organic solvents which may themselves affect the central nervous system.

Drugs that act upon the central nervous system (CNS) after systemic administration have the properties of low ionization at plasma pH, low binding to plasma protein, and high lipid/water partition coefficients (Goldstein et al 1969). Because of their good lipid solubility such drugs are usually poorly soluble in aqueous media unless they are able to form water soluble salts. Alternatively, they may be dissolved in water miscible solvents which, however, such as in the case of nitrazepam (Vieth et al 1968), may exert actions of their own. Incorporation of such drugs in oil-in-water emulsions or solubilization by means of surfactants are other possibilities (Pflegel 1982; Groves 1985) to make them suitable for intravenous (i.v.) administration but these procedures are unsuitable for routine pharmacological investigations. Recently, we were able to demonstrate that three central depressants, methohexitone, midazolam and flunitrazepam, had comparable central actions after i.v. administration as solutions and suspensions to rabbits, when tested by means of the electrocorticogram (Stumpf & Viernstein 1990). In a series of previous studies (Gogolák & Stumpf 1980) it could be shown, however, that the frequency of the bioelectric rhythm elicited in the red nucleus and cerebellum of the rabbit by a variety of central depressants is the most sensible criterion for the level of anaesthesia: a negative linear correlation was found to exist between the frequency of the rubral rhythm and the logarithm of the pentobarbitone concentration in blood (Gogolák 1970). In the present study we used this criterion for an exact comparison of the anaesthesia elicited by methohexitone sodium as a solution and as a suspension.

Materials and methods

Sixteen male rabbits (New Zealand White and Russian-hybrid, Himberg, Austria), 2·3 to 3·3 kg, were used in this study. The method of Monnier & Gangloff (1961) was employed for recording the electrical activity of the red nucleus because this method allows experimentation in conscious, non-curarized animals without pain or discomfort. Methohexitone was given as a suspension (100 mg with 200 mg glycerol and 50 mg acacia, in 10·0 mL isotonic saline) or, in the form of its sodium salt, in equimolar doses, as solution intravenously. The diameter of the particles was measured by means of photon correlation spectroscopy (Brookhaven Instruments Corporation, New York). The values were found to be log-normally distributed with a mean of 750 nm (Fig. 1). The duration of the injection was kept constant at 1 min for both solution and suspension. Each rabbit received both the solution and the suspension in a dose of 10 mg kg^{-1} each with an interval of 5 h between the injections; eight rabbits received the suspension first and the solutions as the second administration while in the remaining eight rabbits the sequence of injections was reversed. Fig. 2 shows both an example of the

Correspondence: H. Viernstein, Institute of Pharmaceutical Technology, University of Vienna, Währinger Strasse 25, A-1090 Vienna, Austria.

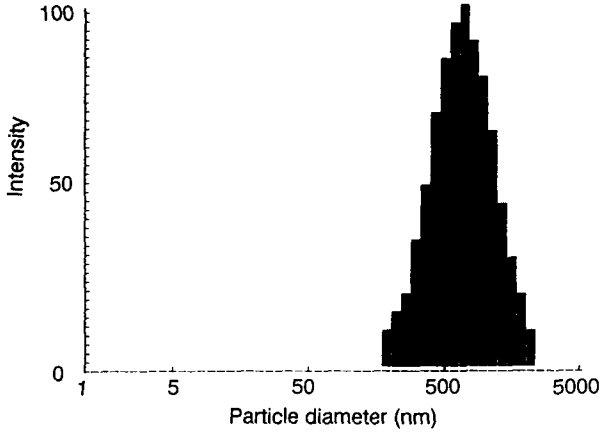

FIG. 1 Plot of methohexitone particle-diameters (nm) vs frequency.

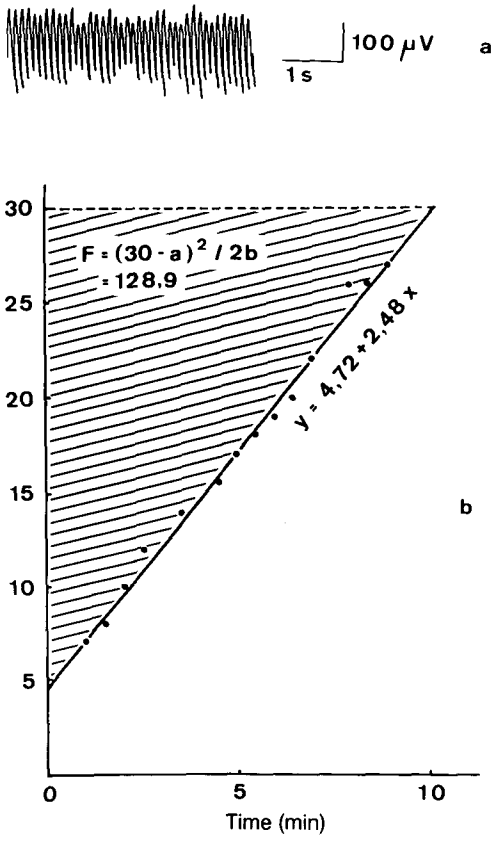

FIG. 2. (a) Example of a rubral rhythmic activity induced by methohexitone. (b) Plot of frequency vs time (min) after injection-from experiment no. 6. For further explanation of AUC (= F) see text.

Table 1. Anaesthesia induced by injection of solutions of methohexitone sodium and suspensions of methohexitone characterized by their AUC values.

Rabbit	Solution	Suspension	AUC Difference	1st	2nd	Difference
1	149·3	186·7	−37·4	186·7	149·3	37·4
2	168·6	132·3	36·3	132·3	168·6	−36·3
3	172·2	200·7	−28·5	200·7	172·2	28·5
4	134·8	190·0	−55·2	190·0	134·8	55·2
5	183·7	159·2	24·5	183·7	159·2	24·5
6	128·9	108·6	20·3	128·9	108·6	20·3
7	116·1	133·0	−16·9	133·0	116·1	16·9
8	133·7	127·7	6·0	133·7	127·7	6·0
9	162·0	126·3	35·7	162·0	126·3	35·7
10	147·3	143·7	3·6	143·7	147·3	−3·6
11	64·0	84·5	−20·5	64·0	84·5	−20·5
12	129·0	151·8	−22·8	151·8	129·0	22·8
13	145·4	124·4	21·0	145·4	124·4	21·0
14	125·7	138·1	−12·4	125·7	138·1	−12·4
15	71·7	89·8	−18·1	89·8	71·1	18·1
16	150·1	102·3	47·8	150·1	102·3	47·8
Mean differences			−1·04			16·34*

1st and 2nd denote the 1st and 2nd administration (with an interval of 5 h between the administrations). *$P < 0.05$.

barbiturate-induced rhythmic bioelectric activity of the red nucleus (a) and the method of the quantitative evaluation (b): the linear regression of the frequency of the rubral rhythm against time was calculated by means of the least-squares-method and the 'area under the curve' (AUC) was determined; 30 Hz was taken as 'baseline' because this is the approximate frequency of the rubral rhythm during wakefulness.

The solubility of methohexitone was determined as follows. Methohexitone (7·5 mg) was suspended in 100 mL phosphate buffer, pH 7·5 and shaken vigorously for 15 min. The amount dissolved was measured and found to be 4·2 mg.

Results

The AUC values from experiments with injected solutions of methohexitone sodium and suspensions of methohexitone are shown in Table 1. Although there is a considerable variation of these values amongst the animals, the mean difference between solution and suspension was not significant ($P > 0.05$). However, the mean difference between the first and second application (16·34) was significant ($P < 0.05$). The former observation suggests the same bioavailability of methohexitone and methohexitone sodium for the CNS; there were also no differences in onset, maximum and duration of action; the latter indicates the development of tolerance.

Discussion

In this study a virtually identical action upon the central nervous system of methohexitone suspension and methohexitone sodium solution after i.v. administration could be demonstrated. As it seems unlikely that particles with a mean diameter of 750 nm can penetrate the blood-brain or the blood-cerebrospinal fluid barrier, it may be assumed that the methohexitone particles are dissolved in the blood before entry into the brain. While there can be no doubt that even in a suspension a small percentage of the drug will be dissolved, it is surprising that the (complete) dissolution should occur fast enough to account both for the onset of action and the bioavailability for the CNS which were the same as after administration of the methohexital sodium solution.

It could be demonstrated (see Materials and methods) that even after 15 min not more than 56% of methohexitone was dissolved. Consequently, it is difficult to understand how this poor solubility could account for the fast onset of action of the suspension.

The large variability of the values between the animals is not surprising. Various genetic and environmental factors such as age, sex, strain, and ambient temperature are thought to affect the response of animals to barbiturates (Vesell 1968). Recently, circa-annual variations in acute toxicity of phenobarbitone in mice were shown to exist (Bruguerolle et al 1988). Although in our experiments there was a decline in the combined solution and suspension AUC values during the course of the study—which was carried out over 5 months—this decline was not significant. On the other hand, an acute tolerance to the central actions of barbiturates, also verified by the electroencephalogram, had been previously shown (Stumpf & Chiari 1965; Stumpf & Viernstein 1990).

Nevertheless, the finding that lipid soluble drugs, injected i.v. in the form of suspensions, act upon the CNS virtually identically to the same drugs administered i.v. in the form of solutions, suggests valid pharmacological investigations can be carried out without the necessity to use organic solvents.

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Partition and distribution coefficients of aryloxypropanolamine β-adrenoceptor antagonists

M. RECANATINI, *Università di Bologna, Dipartimento di Scienze Farmaceutiche, Via Belmeloro 6, 40126 Bologna, Italia*

Abstract—n-Octanol/water partition and distribution coefficients of fifteen β-blockers have been measured and the relationships between log P (neutral species), log P_i (fully ionized species) and log $D_{7.4}$ have been examined. A strict correlation exists among these three parameters, suggesting that the ionization exerts similar effects on the partition behaviour of these drugs.

The aryloxypropanolamines are the most widely represented chemical class within the group of the β-adrenergic blocking drugs. It is known that their cardiac activity is correlated with their lipophilic character and a number of studies have appeared in the literature dealing with this argument (Hellenbrecht et al 1974; Rauls & Baker 1979; Cruickshank 1980; Harada et al 1981; Gortner & Hellenbrecht 1988). The lipophilicity of these drugs has also been shown to have a role in some of their biochemical actions (Street & Walsh 1984), as well as in their binding to tissues (Dax & Partilla 1982; Ijzerman et al 1985, 1987; Bree et al 1986). Schoenwald & Huang (1983) studied the corneal penetration of β-blockers, while the relationships between the pharmacokinetics and the lipophilicity of these drugs in man have been thoroughly reviewed by Hinderling et al (1984). Several papers have been devoted to the study of the partition properties of β-blockers (Woods & Robinson 1981; Barbato et al 1990).

The partition coefficients published in the literature are collected in the database of the Pomona College MedChem Project (1986), which also provides a list of the most reliable data called STARLIST. From the same source a program is available called CLOGP, which allows one to calculate the partition coefficient of a given molecule. Considering the usefulness of a reliable method of calculation of the partition data, it is important to check the performance of the program with sets of carefully measured values. In two recent articles a good correlation between experimental and calculated partition coefficients of a number of β-blockers has been shown (Recanatini 1989; Mannhold et al 1990).

Because of their basic nature, the aryloxypropanolamines are partially ionized at physiological pH (7·4), and the ionization influences the partition behaviour of the compounds. In the following we will use the term partition coefficient (P) to mean the ratio of the concentrations of a single species between the two phases, and the term distribution coefficient (D) to mean the ratio of the concentrations of all the species (i.e. neutral, ionic, ion-pair, ion clusters) between the octanol and aqueous phases.

It is the aim of this paper to present a consistent set of log P and log $D_{7.4}$ data of a number of β-blocking drugs with the aryloxypropanolamine structure. We take into consideration the partition of the compounds both as neutral species and as ionic species; a comparison is made between these values and the distribution coefficients at physiological pH. A comparison between the experimental log P values and those calculated by means of the CLOGP program is also presented, in order to further explore the reliability of the calculated values.

Materials and methods

Chemicals. The following drugs were obtained from the indicated companies: diacetolol hydrochloride (May & Baker, Dagenham, UK); cetamolol hydrochloride (Wyeth-Ayerst, Princeton, USA); moprolol hydrochloride (Simes, Milano, Italy); bisoprolol fumarate (Merck, Darmstadt, Germany); bunitrolol and pargolol (Boehringer Ingelheim, Ingelheim, Germany); befunolol hydrochloride (Thilo, Sanerlach, Germany); metipranolol and carazolol (Boehringer Mannheim, Mannheim, Germany); mepindolol sulphate (Schering, Berlin, Germany); betaxolol (L.E.R.S., Paris, France); procinolol (Roussel Uclaf, Rounainville, France); flusoxosol hydrochloride (Hoffmann-La Roche, Basel Switzerland); penbutolol (Hoechst, Frankfurt, Germany); bornaprolol hydrochloride (Rhône-Poulenc, Gennevilliers, France).

All the substances used for the buffer solutions as well as n-octanol were of analytical grade and were purchased from Farmitalia-Carlo Erba, Milano, Italy.

Methods. All the partition measurements were carried out following the shake flask method (Leo et al 1971). Each reported value is the average of at least four runs, with a standard deviation $\leq 0{\cdot}03$; the amount of substance used in the partition studies was such to allow a tenfold interval of concentration whenever possible. It has to be noted that in no case was a dependence of the log $D_{7.4}$ values on the solute concentration found, which suggests that ion-pairing does not influence the partition behaviour.

The partition coefficients of the neutral compounds (log P) were measured using 0·1 M NaOH as aqueous phase (pH = 13), while those of the ionized molecules (log P_i) were measured using 0·1 M HCl (pH = 1·2); the potassium dihydrogen phosphate/sodium hydroxide buffer was used for the measurements at pH 7·4 (log $D_{7.4}$). The concentrations of the substances used were such that the resulting ionic strength of the solutions ranged between 0·1 and 0·3. Ionic strength below 0·1 had a negligible