The osmotic minipump: a new tool in the study of steady-state kinetics of drug distribution and metabolism

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The distribution of a drug and its metabolites to different tissues in the organism is based on a number of absorption and elimination rate constants. A perfect steady-state distribution can only be reached if the drug is released in the organism with a constant rate for a prolonged period of time. A limitation to conventional dosage forms is that they release drugs at time-varying rates (Yates, Benson & others, 1975). Recently devices have been described that release drugs with a constant rate (Theeuwes & Yum, 1976; Scheu, Sperandio & others, 1977; Madan & Shanbag, 1978). One of these devices, the ALZET™ osmotic minipump was shown to have a constant delivery rate *in vitro* for periods up to 1 week (Theeuwes & Yum, 1976). This osmotic minipump consists of a 0·17 ml collapsible reservoir of flexible, impermeable material, surrounded by a sealed layer containing an osmotic agent. This system is contained by a semi-permeable membrane that controls the rate at which the osmotic agent imbibes extracellular fluid if the pump is placed subcutaneously in experimental animals (further details: Theeuwes & Yum, 1976). We report here on the chronic *in vivo* delivery of propranolol in rats and the subsequent tissue distribution of this drug and its metabolites.

We used ALZET™ osmotic minipumps (batch AR-C507) with a pumping rate of approximately 0·7 μl h^{-1} at 37°. In a preliminary experiment we investigated the release characteristics of 4 of these minipumps *in vitro*. The pumps were filled with a 0·9% NaCl solution containing (±)-propranolol in a concentration of 41·2 mg ml^{-1} (equivalent to 7 mg/0·17 ml). (±)-[^{3}H]Propranolol (randomly labelled in the naphthylgroup) was added to a specific activity of 3·3 mCi mmol^{-1}. Ascorbic acid was added as an antioxidant. The minipumps were placed in a 0·9% NaCl solution at 37°. Total radioactivity released in each 24 h was measured by collecting the fluid released daily in glass tubes and subsequent liquid scintillation counting. Fig. 1 shows the cumulative release rate of propranolol by these minipumps. During the first 9 days propranolol was released linearly at a rate of 0·37 (mean) s.d. 0·01% of total content h^{-1} (or 8·9 s.d. 0·3% per day). At the end of this period 80% of the total content was released. Another 10% was released more slowly during the next 6 days.

To test whether propranolol was chemically stable in the pumps, a portion of the fluid released on day 9 from each of the pumps was subjected to a propranolol-specific extraction procedure modified from Black, Duncan & Shanks (1965). Briefly, the solution released was diluted with 2 ml 0·9% NaCl. 50 μl of 10M NaOH were added (pH > 13) as well as 1 g NaCl. This mixture was shaken for 15 min with 3 ml heptane containing 1·5% isoamylalcohol. After centrifugation at 1600 *g* for 5 min at 4°, 2 ml of the heptane phase was added to 15 ml Aquasol scintillation fluid. More than 98% of total radioactivity was contained in the heptane phase. Moreover, only one radioactive spot with the same R_F value as that of propranolol standard could be detected after thin-layer chromatography with a solvent system of butanol–acetic acid–water (40 : 10 : 5). These results show that propranolol is chemically stable in the minipumps for at least 9 days.

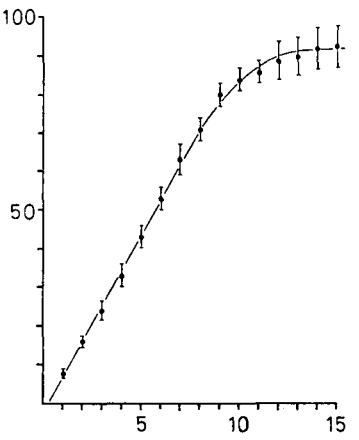

FIG. 1. Release of (±)-propranolol by osmotic minipumps *in vitro*. Ordinate: Cumulative (±)-propranolol released (%). Abscissa: time (days).

In vivo experiments were performed in 5 male spontaneously hypertensive Wistar rats (T.N.O. Proefdierenbedrijf, Zeist), 200–250 g. This strain was chosen because of haemodynamic studies that were performed parallel to the pharmacokinetic experiments. Under ether anaesthesia a catheter was implanted through the right jugular vein into the right atrium to allow the collection of venous blood. Pumps were filled with a solution containing a concentration of (±)-propranolol equivalent to a release rate of 1 mg kg^{-1} per 24 h on the basis of the *in vitro* calibration. A tracer amount of (±)-[^{3}H]propranolol was added to a specific activity of 3·3 mCi mmol^{-1}. After implan-

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tation of the minipumps, rats were placed in metabolic cages to collect urine and faeces daily. Blood samples of 0·5–1 ml were taken on day 1, 3 and 6 after the start of the infusion. Heparinized blood samples were centrifuged at 1600 *g* for 10 min at 4°. Erythrocytes were lysed in 5 ml distilled water. Daily faeces were homogenized in 20 ml 0·9% NaCl. A portion of plasma, erythrocytes, daily urine and faeces homogenates were analysed for total radioactivity content by quantitative combustion (Packard 306 sample oxidizer). Another portion was assayed for propranolol according to the extraction procedure described above. Quenching was corrected for by internal standardization.

Table 1 shows plasma and erythrocyte lysate concentrations of propranolol and its metabolites on day 1, 3 and 6. Both in plasma and erythrocytes the concentration of propranolol and its metabolites remains constant throughout the period of 6 days. Total metabolite concentration is 8–10 fold higher. This agrees with previous studies in experimental animals and man in which it was shown that propranolol is metabolized extensively (Paterson, Connoly & others, 1970; Hayes & Cooper, 1971).

In Table 2 the daily excretion of propranolol and its metabolites in urine and faeces is given. At the end of the first day only 39% of the dose infused during that day was recovered. Thereafter recovery was 80% or more. Most of the radioactivity recovered was excreted in the urine as metabolites. Urinary metabolites accounted for 73–85% each day, except on day 6. On this day a relatively high production of faeces caused a faecal metabolite excretion of 43% of the total excretion. Propranolol metabolite excretion in the faeces on the other days was 13–24%. This agrees quite well with the results of Hayes & Cooper (1971) who found that rats excrete approximately 25% of a single dose of propranolol via the faeces as metabolites. Both plasma and excretion data show that a good steady-state was obtained with the minipumps from day 1 to day 6. The rapidity with which this steady-state is reached agrees with the relatively short plasma half-life of 82 min of propranolol in this species (Struyker-Boudier, Smits & van Essen, 1978).

Finally, rats were killed on day 6 to study steady-state distribution of propranolol and its metabolites in a number of tissues. These tissues were weighed and homogenized in 10–20 ml 0·9% NaCl. A portion of each homogenate was combusted to determine total radioactivity content. Another portion was assayed for propranolol according to the extraction procedure previously mentioned. Steady-state distribution data for a number of organs are given in Table 3. Relatively high propranolol concentrations were found in the lungs, whereas the heart, kidneys, testes and brain contained concentrations comparable to those in plasma. Highest metabolite concentrations were detected in liver, kidneys and certain parts of the brain, i.e. brainstem, hypothalamus and hippocampus. Garvey & Ram (1975) also reported high concentrations of propranolol metabolites in these areas of the brain after repeated subcutaneous injection of this agent in the spontaneously hypertensive rat. The chemical nature of this metabolite and its possible significance in the mode of action of propranolol remain to be elucidated.

Table 1. *Propranolol and total metabolite concentration in plasma and erythrocytes lysate (ng ml^{-1}) on day 1, 3 and 6 after the start of minipump infusion of propranolol 1 mg kg^{-1} per 24 h. Mean values as well as the range (in parentheses) are given for 5 rats.*

	Plasma		Erythrocytes lysate	
	Propranolol	Metabolites	Propranolol	Metabolites
Day 1	32 (13–54)	257 (126–519)	19 (9–30)	153 (130–167)
Day 3	25 (16–31)	418 (129–887)	10 (7–14)	417 (113–853)
Day 6	39 (13–101)	300 (193–513)	14 (5–35)	401 (264–665)

Table 2. *Daily excretion of propranolol and its metabolites in urine and faeces.* Total recovery is given as percentage recovered of the amount of d min^{-1} infused during that day. The other columns give the relative amounts excreted as propranolol and metabolites in urine and faeces. Means ±s.e.m. for 5 rats.

		Urine		Faeces	
Day	Total recovery (%)	Propranolol (%)	Metabolites (%)	Propranolol (%)	Metabolites (%)
1	39·2 ±7·8	2·3 ±0·5	79·3 ±15·4	0·4 ±0·1	18·0 ±3·4
2	80·0 ±8·1	2·0 ±0·2	80·0 ±13·7	0·2 ±0·1	17·8 ±4·1
3	80·5 ±7·4	2·8 ±0·6	79·6 ±14·4	0·2 ±0·1	17·4 ±6·5
4	92·0 ±18·3	1·9 ±0·3	85·4 ±20·3	0·2 ±0·1	12·5 ±8·7
5	105·6 ±26·4	2·6 ±0·4	73·2 ±20·5	0·3 ±0·1	23·9 ±11·4
6	128·7 ±30·5	2·1 ±0·4	54·9 ±16·7	0·4 ±0·2	42·6 ±27·9

Table 3. *Steady-state distribution of propranolol and its metabolites on day 6 after the start of minipump infusion of propranolol 1 mg kg^{-1} per 24 h.* Mean values (ng g^{-1}) as well as the range (in parentheses) are given for 5 rats.

	Propranolol	Metabolite
Plasma	39 (13–101)	300 (193–513)
Erythrocytes	14 (5–35)	401 (264–665)
Heart	42 (16–83)	429 (213–788)
Lungs	206 (94–419)	367 (160–541)
Testes	43 (25–62)	402 (85–1070)
Liver	28 (9–63)	2620 (2180–3280)
Kidneys	54 (20–115)	2600 (2100–3330)
Brainstem	42 (26–81)	871 (545–1330)
Cerebellum	30 (18–64)	446 (319–676)
Hypothalamus	20 (8–77)	722 (380–1230)
Hippocampus	31 (19–72)	579 (266–1150)
Cortex	43 (13–84)	261 (58–461)

In conclusion, the osmotic minipump provides a useful tool for the study of steady-state kinetics of drug distribution and metabolism. A constant *in vitro* release rate by these minipumps is reflected in constant plasma concentrations of this drug and its metabolites throughout the infusion period. Without disturbing the experimental animal for frequent injections a steady-state distribution can thus be accomplished.

April 13, 1978

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The effects of some analgesic and neuroleptic drugs on the spasmogenic actions of substance P on guinea-pig ileum

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The role of substance P (SP) as a neurotransmitter particularly associated with pain sensation at the level of the spinal cord is gradually gaining support (Henry, 1976; Randic & Miletic, 1977). Substance P appears to be stored in vesicles (Cuello, Jessel & others, 1977) and can be released from nervous tissue following depolarization (Iversen, Jessel & Kanazawa, 1976; Otsuka & Konishi, 1976). Inhibition of release of SP from the trigeminal nucleus by opiates and by endorphins (Jessel & Iversen, 1977) suggests that the actions of SP and the endogenous opiates are closely linked to pain perception. However, some reports claim that the action of SP may not be mediated by a unique receptor but possibly via an opiate receptor (Davies & Dray, 1977).

This study was undertaken to determine the anti-SP activity of compounds known to possess analgesic action, on the spasmogenic response to SP in the guinea-pig isolated ileum. It was envisaged that it might be possible to explain the analgesic activity of certain compounds on the basis of SP antagonist activity. The guinea-pig ileum preparation was subsequently used in an attempt to identify a specific antagonist to SP. The results of a recent study by Bury & Mashford (1977) suggest that SP acts directly on the smooth muscle of the guinea-pig ileum, possibly via a SP receptor. In addition, a prejunctional action of SP on cholinergic transmission has been suggested by Hedqvist & von Euler (1975).

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Segments from the caecal end of the guinea-pig ileum were suspended in a 10 ml organ bath containing Krebs-bicarbonate solution gassed with 5% CO_2 in oxygen. Synthetic substance P (Beckman Inc. or Dr J. S. Morley, ICI Alderley Park) was added at 3 min intervals and left in contact with the tissue for 30 s before washing. Test compounds were made up in Krebs-bicarbonate solution unless otherwise stated and were added to the bath solution 2 min before the addition of SP. Contractions were recorded isotonically. Each compound was tested on a fresh piece of ileum obtained immediately after an animal had been killed. Earlier experiments had demonstrated that if the tissue had been allowed to stand in Krebs solution for longer than an hour before use, then the sensitivity of the tissue to SP was initially higher than that observed in tissue from a freshly killed animal. This increased sensitivity did however, gradually subside after repeated regular dosing with SP. Control dose-response curves to SP were defined both before and after that obtained in the presence of the test compound.

At concentrations up to 10 μM the peripherally acting analgesics indomethacin (MSD), paracetamol (Winthrop), ketoprofen (M & B), naproxen (Syntex and ibuprofen (Boots) failed to antagonize the contractions to SP.

The opiate receptor antagonist naloxone (Winthrop), at concentrations up to 15 μM, also failed to modify the contractile responses to SP.

The opiate receptor agonists morphine, codeine, pethidine (Macfarlan Smith), and diethylthiambutene