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Kinetics and distribution of the β-adrenergic agonist salbutamol in rat brain

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The kinetics of the β-adrenergic agonist salbutamol and its distribution in the central nervous system were examined in rats by high-performance liquid chromatography with electrochemical detection. After 10 mg kg^{-1} i.v. salbutamol, the decline in drug plasma concentrations was biphasic and salbutamol was cleared very rapidly with a β-half-life of about 37 min. Salbutamol rapidly penetrated the 'blood-brain barrier' and reached brain concentrations amounting to about 5% of the plasma concentrations. In structures outside the blood-brain barrier (pineal and pituitary glands) the drug achieved concentrations more than 100 times those in whole brain. The results support the hypothesis that stimulation of central β-adrenergic sites is responsible for salbutamol's pharmacological and biochemical effects.

Salbutamol, a β-adrenergic agonist, is reported to have clinical antidepressant activity (Lecrubier et al 1977, 1980; Widlöcher et al 1977; Lerer et al 1981). Furthermore, a number of biochemical (Waldmeier 1981; Sugrue 1982) and pharmacological (Francès et al 1979; Przegalinski et al 1980; Ortmann et al 1981; Borsini et al 1982) studies suggest the compound has central effects. Nevertheless, very little is known about the drug's ability to penetrate the blood-brain barrier and to diffuse into brain tissue. Disposition studies in man have necessarily been limited to the quantitation of salbutamol in plasma and urine (Martin et al 1971, 1976; Oosterhuis & Van Boxtel 1982). Preliminary reports have appeared on tissue distribution of [^{3}H]salbutamol in laboratory animals (Martin et al 1971) but no detailed information is available about this aspect of the drug disposition. The development of a specific and sensitive high-performance liquid chromatographic procedure with electrochemical detection has permitted investigation of the relation of plasma levels and brain distribution of salbutamol in the rat.

Methods

Male CD-COBS rats (Charles River, Italy), ca 200 g, were dosed intravenously with salbutamol sulphate (10 mg kg^{-1}). At various times after injection the animals were killed, blood samples were collected in heparinized tubes, centrifuged and the plasma was stored at −20 °C. Brains and spinal cords were removed immediately and stored at −20 °C. Brain areas were dissected according to Glowinski & Iversen (1966). For extraction, 0·1–0·5 ml plasma was mixed thoroughly with 0·9–4·5 ml of acetone-1 M formic acid (85 : 15 v/v). The supernatant was washed twice with n-heptane-chloroform (4 : 1 v/v). The organic phase was discarded and the aqueous phase was buffered to pH 9 using 1 M sodium hydroxide and 0·5 M phosphate buffer then extracted twice with 5 ml of ethyl acetate. The combined extracts were evaporated to dryness and redissolved in 0·1 the mobile phase containing 50 ng of fenoterol as a marker and 10–20 µl were injected into the hplc column. Brain regions and spinal cords were homoenized (9 ml g^{-1}) in cold-acetone-1 M formic acid, centrifuged and then processed as described for plasma.

Salbutamol was determined on a Waters Chromatograph (Waters Associates Inc., Milford, MA, USA) equipped with a model U6K universal liquid injector, a model 6000A solvent delivery system and a reverse phase column (µ Bondapak C18, 3·9 mm × 30 cm) at room temperature (22 °C). The mobile phase was a mixture of sodium phosphate buffer (0·06 M)–acetonitrile–methanol (78 : 4 : 18 v/v), pH 7·4 at a flow rate of 1·0 ml min^{-1}. Compounds eluted from the column were detected by an electrochemical detector with a glass carbon electrode (Bioanalytical Systems, West Lafayette, In.). The working electrode potential was set at +0·90 V. Representative chromatograms of brain samples are shown in Fig. 1. Retention times were 8·0 min and 17·5 min for salbutamol and fenoterol respectively.

Standard curves were prepared daily using known

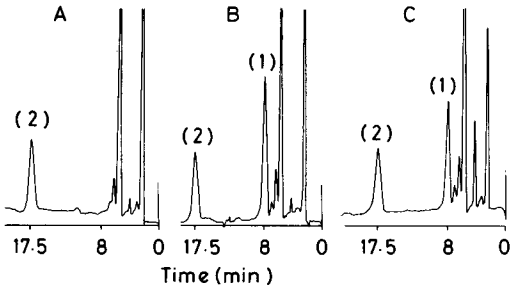

Fig. 1. Hplc chromatograms of extracts from (A) drug-free brain, (B) brain spiked with salbutamol, (c) brain of rats intravenously injected with salbutamol sulphate (10 mg kg^{-1}). Peaks: salbutamol (1) and fenoterol (2).

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concentrations of salbutamol. The peak area ratio (salbutamol to internal standard) was plotted versus the added concentrations of salbutamol. The slope of this curve, determined by linear regression analysis, was used to calculate salbutamol concentrations in unknown samples.

Mean recovery of salbutamol from control plasma was 89·9 ± 9·3% and from brain homogenate 85·4 ± 4·1% (mean ± s.d.). The calibration curves were linear in the range 1 ~ 10 ng per injection. Additional sample dilution was necessary for concentrations higher than 10 ng per injection. 1 ng per injection was the detection limit, corresponding to 5 ng ml^{-1} of plasma or g^{-1} of brain.

The kinetic data were calculated using a weighted non-linear, least square fitting program on a HP 85 computer.

Results and discussion

The plasma and brain concentration-time curves of salbutamol after i.v. injection (10 mg kg^{-1}) to rats are shown in Fig. 2. The concentration data at each time represent the mean of 4 animals. In these experimental conditions the decline in plasma concentrations was biphasic and the drug was cleared very rapidly with a β-half-life (t½ β) of 36·7 min. The central volume of distribution (Vc) and the steady state volume of distribution (V_{ss}) were 0·15 and 0·95 litre kg^{-1} respectively. Total body clearance (Cl_s) was 39·2 ml min^{-1} kg^{-1}. Brain concentrations of salbutamol did not show a discernible accumulation phase. Maximum concentrations (C_{max}) were reached 1 min after salbutamol, representing about 3% (0·83 ± 0·10 μg g^{-1}) of the plasma concentrations (31·54 ± 5·08 μg ml^{-1}). Hence the brain to plasma concentrations ratio showed a small increase over the first 15 min then remained relatively constant at the ratio of 0·05 : 1. Brain t½ β (28·2) was comparable to the plasma t½ β. Thus, brain area under the curve (AUC) was 11·10 μg g^{-1} × min, about 3·9% of the plasma AUC (284·39 μg ml^{-1} × min).

The distribution of salbutamol in brain regions and spinal cord was investigated 30 and 90 min after i.v. injection. Among the brain areas considered the hippocampus showed the highest concentration (0·13 ± 0·01 μg g^{-1} ± s.e.) and the lowest concentration was detected in the striatum (0·06 ± 0·01 μg g^{-1} ± s.e.), soon after i.v. injection (see Table 1). No marked change in brain area distribution was apparent at later times (90 min) and the decline in salbutamol concentrations was similar to that in the whole brain. At both intervals considered, the spinal cord showed salbutamol concentrations of about three times the whole brain concentrations. The highest concentrations of salbutamol, however, were achieved in structures outside the 'blood-brain barrier' (pituitary and pineal glands). In these regions the drug reached concentrations more than 100 times those in whole brain. Furthermore, at both times considered the plasma concentration ratios

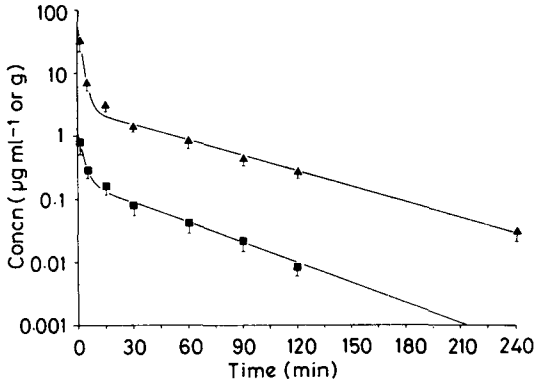

FIG. 2. Plasma (△) and brain (□) concentration-time curves of salbutamol in rats intravenously injected with salbutamol sulphate (10 mg kg^{-1}). Each point is the mean ± s.e.m. of 4 determinations.

were about 6 : 1 and 8 : 1 respectively for the pituitary and pineal glands, indicating preferential accumulation and retention of salbutamol by these structures.

In conclusion our findings indicate that, after i.v. injection, salbutamol rapidly penetrates the blood-brain barrier and reaches brain concentrations amounting to about 5% of the plasma concentrations. Although the elimination t½ of the drug in the rat (about 30 min) is short, the relatively high concentrations (about 1 μg g^{-1}) found in rat brain 1 min after i.v. injection of large doses support the hypothesis that stimulation of central β-adrenergic sites is responsible for the drug's pharmacological and biochemical effects.

This agrees with the observation that small amounts of salbutamol (3–30 μg), injected intraventricularly, have clear central action such as potentiating the behavioural effects of 5-hydroxytryptophan (Ortmann et al 1981) and increasing 5-hydroxytryptamine metabolism (Waldmeier 1981; Sugrue 1982). Whether significant brain salbutamol concentrations are achieved after

Table 1. Distribution of salbutamol in brain regions and spinal cord of rats after intravenous injection of 10 mg kg^{-1} salbutamol sulphate.

	Minutes after injection	
Tissue	30	90
Spinal cord	0·25 ± 0·04 (0·17)	0·07 ± 0·01 (0·17)
Cortex	0·08 ± 0·01 (0·05)	0·03 ± 0·01 (0·06)
Striatum	0·05 ± 0·01 (0·03)	0·02 ± 0·005 (0·03)
Brain stem	0·11 ± 0·01 (0·08)	0·03 ± 0·005 (0·06)
Hippocampus	0·13 ± 0·01 (0·09)	0·02 ± 0·01 (0·05)
Cerebellum	0·12 ± 0·02 (0·08)	0·02 ± 0·01 (0·05)
Pituitary gland	9·26 ± 2·03 (6·27)	2·69 ± 0·22 (5·98)
Pineal gland	10·39 ± 0·16 (7·19)	4·02 ± 0·93 (8·93)

Each value (μg g^{-1} ± s.e.) is the mean of 3 determinations (pool of 3–6 rats).

Tissue to plasma concentrations ratio are shown in parentheses.

i.p. or oral doses, however, remains to be established. Study of the regional distribution indicates that salbutamol markedly accumulates in regions outside the blood-brain barrier such as the pituitary and pineal glands. This might be relevant to possible sites of action of the drug which could influence these structures, thereby modifying the central events.

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Quinidine-induced rise in ajmaline plasma concentration

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A high-performance liquid chromatographic method is described for the simultaneous determination of ajmaline and quinidine in human plasma. With 0·5 ml plasma samples of ajmaline and quinidine, concentrations as low as 0·001 and 0·01 μg ml^{-1}, respectively, could be detected and the technique could be used to investigate the effect of quinidine on the pharmacokinetics of ajmaline. Four healthy subjects were given oral ajmaline (50 mg) alone or in combination with quinidine sulphate (200 mg) on separate occasions. When ajmaline was administered alone, its plasma concentrations were less than 0·03 μg ml^{-1}. Quinidine induced a marked increase to give a mean peak concentration of ajmaline which increased from 0·018 μg ml^{-1} after a single administration to 0·141 μg ml^{-1} in combination with quinidine. The area under the ajmaline concentration-time curves was increased 10 to 30-fold by the concurrent administration of quinidine. According to the one compartment open model, the absorption rate constant of ajmaline did not change appreciably, but the elimination rate constant was reduced to approximately 50% of the value in the absence of quinidine. The results indicate the existence of a significant interaction between oral ajmaline and quinidine.

The routine determination of ajmaline is hampered by the low sensitivity, time consumption and poor specificity of the available methods (Kleinsorge & Gaida 1961; Dombrowski et al 1975; Clemans et al 1977). Therefore, its pharmacokinetic properties have not been well understood (Kleinsorge & Gaida 1962: Dombrowski et al 1975; Spilker et al 1975; Iven 1977; Anttila et al 1978) and nothing is known about its kinetic behaviour in the clinical situation, where it is often used in combination with agents such as quinidine.

A specific and sensitive high-performance liquid chromatographic (hplc) method for the determination of ajmaline permits measurement of both ajmaline and quinidine in plasma simultaneously. This technique has been used to investigate the effect of quinidine on the pharmacokinetics of ajmaline given orally to healthy volunteers.

Materials and methods

Ajmaline was supplied by Sigma (St Louis, USA), quinidine was obtained from Nakarai Chem. Ltd (Kyoto, Japan) and quinidine ethylcarbonate, used as the internal standard, was purchased from Hoei Yakko (Osaka, Japan). Standard solutions of ajmaline and quinidine (1·00 mg ml^{-1}, respectively) were prepared weekly in 2% acetic acid and stored at 4 °C. The internal standard solution of quinine ethylcarbonate in diethyl ether was prepared daily and protected from light.

Acetonitrile (Kantokagaku, Tokyo, Japan) and glacial acetic acid (Wako, Osaka, Japan) was used without further purification. All other chemicals were analytical grade products available commercially.

A Shimadzu Model LC-3A high performance liquid chromatograph equipped with a Shimadzu RF-500LC fluorescence spectromonitor and a Zorbax CN column

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