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THE PHYSIOLOGICAL DISPOSITION OF 2-(2,6-DICHLOROANILINO)-2-IMIDAZOLINE (St-155)*

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Abstract—St-155 [2-(2,6-dichloroanilino)-2-imidazoline] is a new hypotensive agent effective in microgram doses. The physiological disposition of the compound in the rat was examined by using ^{14}C-labeled drug and a selective extraction procedure. St-155 was found to be localized reversibly in all of the tissues examined, but especially in the submaxillary gland. The gland to plasma concentration ratio was about 40. The concentration in saliva was lower than that for the plasma, indicating that the compound had accumulated in the gland itself. Examination of the uptake of St-155 into submaxillary slices *in vitro* showed that the accumulation reflected the concentration of non-ionized compound in the bath fluid, suggesting that passage of the drug from plasma to saliva occurs by diffusion of this form of St-155 through the gland.

St-155 (FIG. 1) IS A NEW imidazoline derivative that produces hypotension, sedation and bradycardia, and reduces salivary secretion in man in microgram doses.[1] This report described studies of its physiological disposition in rats and cats, uptake into tissue slices and binding to tissue homogenates, as an adjunct to an investigation of its pharmacology in animals. Particular consideration is given to the localization of St-155 in submaxillary gland and saliva, as part of a more extensive investigation of such localization of drugs.[4]

St-155

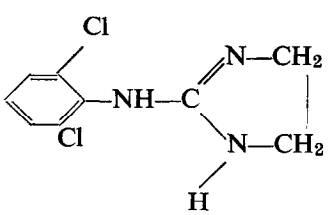

FIG. 1. Structure of 2-(2,6-dichloroanilino)-2-imidazoline (St-155).

METHODS

Procedures in animals (*experiments* in vivo). Male Sprague–Dawley rats weighing 150–200 g were given an i.v. injection of ^{14}C-St-155 (Sp. act. = 12·4 mc/m-mole) labeled in the 2-position of the imidazoline ring. For tissue distribution studies, the animals were lightly anesthetized with ether and 4 ml blood was collected by cardiac

* St-155 is the code number for Catapres, marketed by Boehringer Ingelheim Gmb. H.

puncture and added to 0·1 ml of 5% (approx.) sodium citrate solution. Tissues were immediately removed and homogenized with 9 vol. of 0·1 N HCl solution in a Sorvall Omni-Mixer. Plasma was separated from the blood samples and mixed with 1 vol. of 0·1 N HCl. Protein was precipitated from the plasma mixtures and homogenates by the addition of 0·05 ml of 60% perchloric acid per 10 ml homogenate. The mixtures were centrifuged at 110 *g* for 20 min and 1- or 2-ml samples of the supernatant layers were assayed for St-155 as described below.

Urine from pretreated and control animals was collected overnight in a bottle containing a few drops of 0·1 N HCl. Aliquots of 1 or 2 ml of the urine were assayed as described below.

Cat submaxillary gland preparation. A cat (3·6 kg) was anesthetized with pentobarbital (250 mg). Carbachol was infused (0·2 μg/min) with a cannula in the left femoral vein. Blood samples were taken from the right femoral vein which had been cannulated for injection of heparin (4 mg) and St-155. Saliva from the submaxillary glands was collected by means of cannula inserted into the ducts.

Binding to plasma proteins and tissue homogenates. Tissue binding of St-155 was measured by the centrifugal ultrafiltration of undiluted plasma or of tissue homogenates.[3, 4] The ultrafiltrate ("free" drug) and the homogenate ("total" drug) were assayed for the drug and the concentration of bound drug was determined by difference. The results were expressed as per cent binding.

Uptake by tissue slices. Rats were killed by a blow on the head. The tissues were removed and washed free of blood by brief immersion in ice-cold Krebs–Ringer bicarbonate solution (pH 7·4). Slices 0·5 mm thick were cut with a Stadie–Riggs microtome. About 300 mg of tissue slice was placed in a 30-ml beaker together with 10 ml Krebs–Ringer solution buffered (0·05 M) with Tris, bicarbonate or acetate. The beakers were shaken in a Dubnoff metabolic shaker in an atmosphere of O_2. In some experiments 0·5-ml samples of the bath fluid were removed at intervals during the incubation and assayed for St-155; in others, the incubation was stopped by removal of the slices, which were blotted on filter paper and weighed. Both the medium and the tissues were then assayed for the drug.

Extraction and estimation of St-155. In binding experiments 0·5-ml samples of the homogenate and ultrafiltrates were examined by liquid scintillation spectrometry for content of ^{14}C-St-155 directly. In the experiments *in vivo*, the compound was separated by solvent extraction.

A 1- or 2-ml aliquot of a fluid or tissue homogenate was pipetted into a 50-ml glass-stoppered centrifuge tube containing 1 ml of 10% Na_2CO_3 solution and 25 ml ethylene dichloride. The mixture was shaken for 10 min, centrifuged at 100 *g* for 10 min and then 20 ml of the organic layer was transferred to a second glass-stoppered centrifuge tube containing 1 ml of 0·1 N HCl. The second tube was shaken, centrifuged and a 0·5-ml sample of the aqueous layer was examined for St-155 content by liquid scintillation spectrometry* of the ^{14}C-labeled compound.

After addition of known amounts of St-155 to the homogenate of submaxillary gland, recovery of the drug was 62·1 ± 3·9 (mean per cent ± S.E.) in four determinations. Recovery from heart, muscle, brain, stomach, plasma, urine and saliva varied from 80·6 to 90·1 per cent.

* The liquid scintillation fluid used was prepared from BBOT (2,5-bis-(5-*tert*-butylbenzoxazolyl) thiophene), 0·4%; naphthalene, 0·8%; and methylcellosolve, 40%, in toluene.

For gas–liquid chromatography (GLC) studies, 0·8 ml of the 0·1 N HCl extract obtained above was made alkaline with 1 ml of a 10% sodium carbonate solution and the compound was extracted into 5 ml ethyl acetate with shaking followed by centrifugation. A 4-ml sample of the organic layer was separated and the solution was evaporated to dryness at room temperature in a stream of nitrogen.* The residue was redissolved in 100 μl ethyl acetate, and 1- to 10-μl samples were examined by GLC. A Glowall Chromalab 310 gas chromatograph was used with an Sr^{90} ionization detector set at 10V, the optimum voltage for electron capture of the compound. The coiled glass columns (i.d., 3·5 mm) were packed with either a) 7% SE 33 on Gas Chrom P (80–100 mesh) (6 ft) or b) 2% SE 33 and 1% EGSPZ on Gas Chrom P (80–100 mesh).† The columns, flash heater and detector were operated at 195° 250° and 250° respectively. The carrier gas was argon (30 psi at the beginning of the column). The minimum sample of St-155 detectable in the gas chromatograph was 2–3 ng using the 3 ft column. Adsorption of the compound in the 6 ft column limited the quantitative usefulness to a sample of about 500 ng. The GLC responses were assessed by weighing the chart paper covered by the peak area. The response to standard amounts of St-155 was not linear, so the response to unknown quantities was compared with a standardization curve.

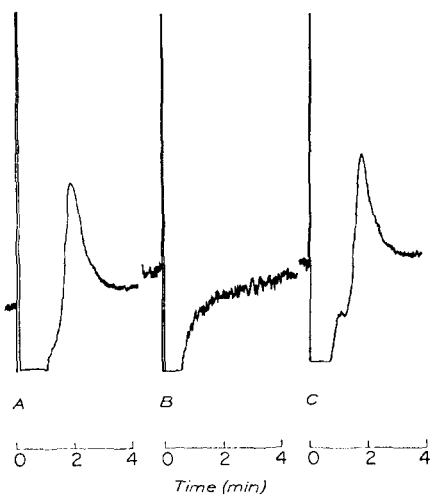

FIG. 2. GLC of St-155 (A) and "blank" (B) and "experimental" tissue extracts (C) (column: 2% SE 33/1% EGSPZ; see text for conditions). (A) Standard, prepared from St-155 dissolved in a mixture of triethylamine, ethyl acetate and water (St-155 base, equivalent to 5 μg St-155 hydrochloride in 10 μl); (B) Plasma extract (6 μl) from control rats prepared as described in Methods section; (C) Plasma extract (6 μl) from rats pretreated with St-155.

Typical chromatograms are shown in Fig. 2. In a "tissue blank" experiments, 8 g of each of the tissues to be examined, and urine, from control rats, was homogenized and extracted as described above. No response was seen in the detector from

* Decomposition of St-155 to products with different GLC and ethylene dichloride/buffer solution partition characteristics was observed when ethylene dichloride extracts were evaporated to dryness on a steam bath. This was prevented by evaporation of solutions at room temperature, under nitrogen, in the absence of NaOH. Aqueous solutions at pH values 3–8 were stable for at least 6 hr at 37°.

† GLC materials were obtained from Applied Science Laboratories, Philadelphia, Pa.

any of the extracts when examined at maximum sensitivity in the gas chromatograph. Equivalent extracts from plasma and tissues of rats pretreated with St-155 (500 μg/kg) showed the peak of the compound (Fig. 2).

Evidence for the specificity of the extraction methods. Extracts of tissues of animals pretreated with the drug (500 μg/kg) showed a single peak in the gas chromatograph with the same retention time as that of authentic St-155 (Fig. 2).

A comparison of GLC and liquid scintillation spectrometric assays demonstrated the specificity of the extraction procedure for St-155, since a near-constant specific activity, defined as a spectrometer response:GLC response ratio, was obtained when aliquots of various extracts were examined by the two techniques (Table 1).

TABLE 1. SPECIFIC ACTIVITIES OF ST-155 IN EXTRACTS OF TISSUES*

Material	Per cent of material extracted into ethyl acetate	Sp. act. in ethyl acetate extract (cpm/μg)
Solution of an authentic sample of St-155	86	136
Heart extract	87	143
Urine extract	78	143
Muscle extract	84	133
Plasma extract	89	129
Brain extract	87	144
Stomach extract	83	142
Submaxillary gland extract	83	145

* Extracts of various tissues from rats pretreated with St-155 and an aqueous solution of the drug were alkalinized with Na_2CO^3 and extracted with ethyl acetate as described under Methods. The partition coefficient of the radioactivity into ethyl acetate was determined for each solution and samples of the final ethyl acetate solutions were examined by liquid scintillation spectrometry and by GLC.

RESULTS

Urinary excretion of St-155 in rats. Approximately 40 per cent of the injected radioactivity was excreted in the urine in 72 hr after intraperitoneal injection of 500 μg/kg of ^{14}C-St-155. The urine was fractionated into water-soluble and ethylene dichloride-soluble fractions by the extraction procedure described earlier. The water-soluble fraction could not be extracted into ethylene dichloride, chloroform or butanol at any pH. The radio-activity in the ethylene dichloride, representing 35 per cent of the total urine radioactivity, was back extracted into 0·1 N HCl and subsequent extraction experiments indicated that it was all ^{14}C-St-155.

Distribution of St-155 in Rat tissues. The concentrations of St-155 in various tissues at ½, 1, 2 and 4 hr after intravenous administration of 250 μg/kg (Table 2, Fig. 3) indicated an apparent volume of distribution of 12·5 times the body weight.* Among tissue levels examined, submaxillary gland had the highest concentration. All of the

* The apparent volume of distribution of St-155 was calculated from the formula

$$\frac{\text{Total body level of St-155 (mg/kg) at } t_0}{\text{Theoretical plasma level of St-155 (mg/kg) at } t_0}$$

and expressed as a multiple of the body weight which the entire dose would occupy at the plasma level calculated. The theoretical plasma level at t_0 was calculated from a graph of log plasma level against time extrapolated back to t_0.

tissue levels declined exponentially. The half-lives in plasma and submaxillary gland were approximately equal (approx. 3¼ hr) while the half-lives in stomach, heart, brain and muscle were shorter (approx. 2 hr).

TABLE 2. TIME COURSE OF TISSUE DISTRIBUTION OF ST-155 IN THE RAT AFTER I.V. ADMINISTRATION OF 250 μg/kg

Tissue	Concentration of St-155 (μg/g-wet wt.)*			
	½ hr	1 hr	2 hr	4 hr
Submaxillary gland	0·75 ± 0·20 (3)	0·73 ± 0·08 (5)	0·65 ± 0·12 (3)	0·39 ± 0·10 (3)
Stomach (free of contents)	0·45 ± 0·10 (4)	0·27 ± 0·03 (6)	0·16 ± 0·02 (4)	0·08 ± 0·01 (4)
Heart	0·21 ± 0·04 (3)	0·13 ± 0·02 (5)	0·14 ± 0·04 (2)	0·06 ± 0·01 (3)
Brain	0·20 ± 0·02 (4)	0·12 ± 0·01 (5)	0·11 ± 0·01 (2)	0·04 ± 0·005 (3)
Muscle	0·13 ± 0·04 (4)	0·12 ± 0·01 (6)	0·08 ± 0·01 (3)	0·04 ± 0·003 (4)
Plasma	0·031 ± 0·007 (3)	0·031 ± 0·002 (3)	0·025 ± 0·007 (3)	0·016 (1)

* Each value is the mean of the number of experiments indicated in parentheses ± S.E.

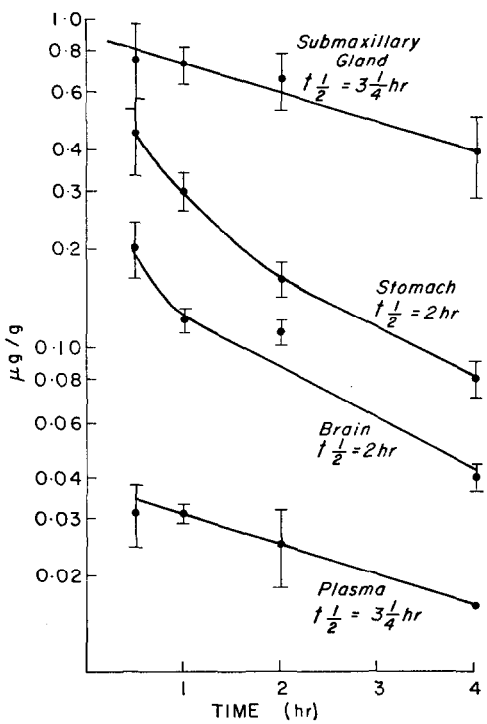

FIG. 3. Levels of St-155 in selected rat tissues after intravenous administration of 250 μg/kg.

Excretion of St-155 in saliva. At intervals after injection of 250 μg/kg of St-155 into an anesthetized cat, saliva levels of the drug were found to be consistently lower than plasma levels (Fig. 4). Assays of plasma, submaxillary gland and saliva by counting of total radioactivity and of ethylene dichloride-extractable radioactivity indicated

that all the radioactivity was extractable into ethylene dichloride and was present as the original compound (see specificity data). The ratio of concentration of non-ionized St-155 (pK_a 7·6) unbound in plasma (pH 7·4) to that in saliva (pH 8·0), calculated by correcting for the differences in plasma and salivary pH was 0·95 ± 0·14 (S.E.) in six determinations.

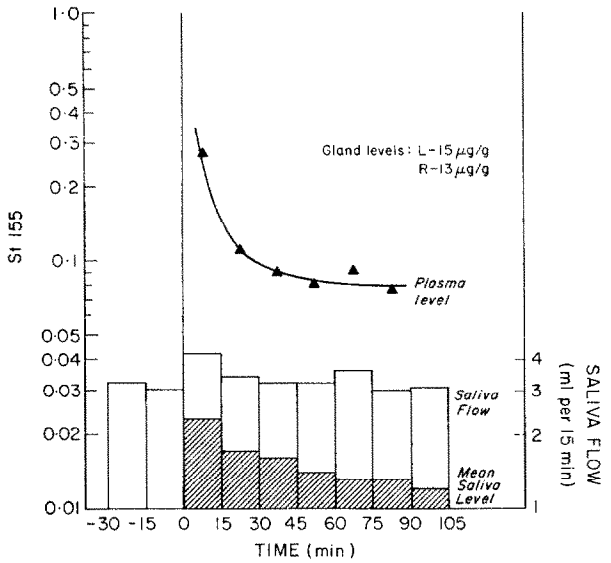

FIG. 4. Levels of St-155 in plasma, submaxillary gland and submaxillary gland saliva of a cat after i.v. administration of 250 μg/kg. (Ordinate: μg/ml.)

TABLE 3. BINDING OF ST-155 TO PLASMA PROTEINS

Concentration of S-155 in plasma* (μg/ml)	Per cent binding
0·28	44 ± 4 (3)
0·10	52 ± 0 (2)
0·05	62 ± 7 (2)
0·01	52 ± 4 (2)
0·005	57 ± 1 (2)

* St-155 was added to whole plasma (5 mg protein/ml) at varying concentrations at room temperature and equilibrated for 1 hr. The plasma was subjected to ultrafiltration through a Visking cellulose membrane. The filtrate (free) and remaining plasma (total) were assayed for St-155. The percent of drug bound (free + bound = total) is given below as the mean (± range) of figures obtained in the number of filtrations indicated in parentheses.

Binding of St-155 to plasma proteins and to tissue homogenates. Binding of St-155 to plasma proteins (Table 3) varied from 44 to 62% over a wide range of St-155 concentrations, Binding to homogenates (10%, w/v) of rat tissues in Krebs–Ringer solution (pH 7·4) was highest for submaxillary gland (Table 4). Binding to various concentrations of submaxillary gland homogenates in normal saline indicated that

binding to a theoretical 100 per cent homogenate would be 57 per cent (Fig. 5).[3, 4] Binding to the submaxillary gland homogenates in Krebs–Ringer solution and in normal saline were similar.

TABLE 4. BINDING OF ST-155 TO HOMOGENATES OF SELECTED RAT TISSUES

Tissue*	Per cent bound
Submaxillary gland	29·9 ± 0·0 (2)
Stomach	26·0 ± 0·5 (2)
Heart	28·2 ± 0·0 (2)
Brain	25·4 ± 2·8 (3)
Muscle	14·9 ± 1·8 (3)

* Homogenates of rat tissues (10%, w/v), prepared in Krebs–Ringer solution (pH 7·4) containing St-155 (0·28 μg/ml), were were subjected to ultrafiltration through a Visking cellulose membrane.

Uptake of St-155 by tissue slices. St-155 was taken up rapidly by tissue slices incubated areobically in Krebs–Ringer bicarbonate solution and reached a steady state distribution between the slice and the medium (Fig. 6, Table 5). The slice: medium

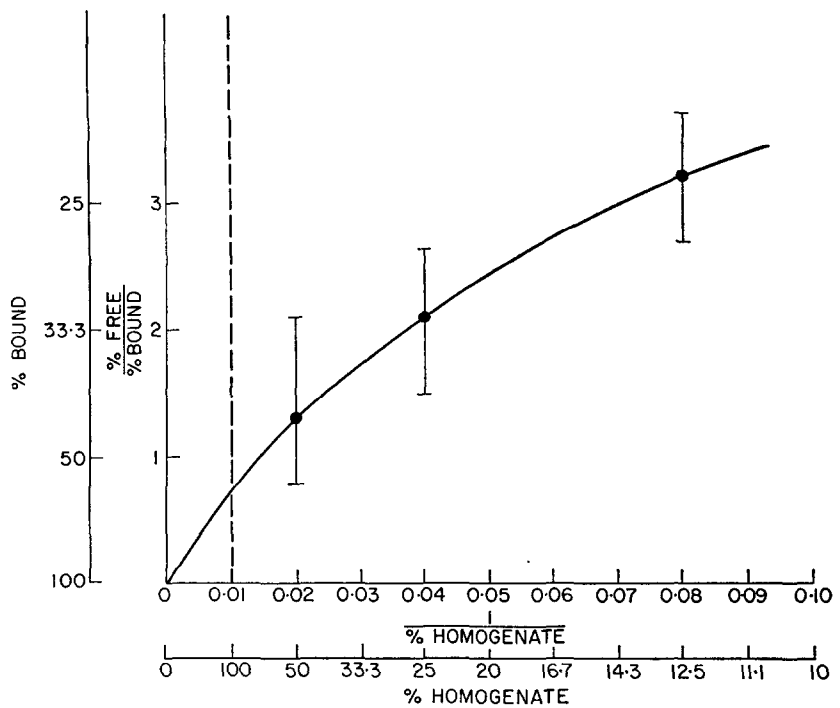

FIG. 5. Binding of St-155 to submaxillary gland homogenates. Homogenates of submaxillary gland in normal saline (pH 6·2) containing S-155 were examined as described in Table 4. Each point is the mean (± range) of 2–3 determinations.

(S/M) ratio was highest for submaxillary gland slices, and for this tissue the pH dependence of the ratios reflected the varying degrees of ionization of St-155 in solutions of different hydrogen ion concentration (Table 6).

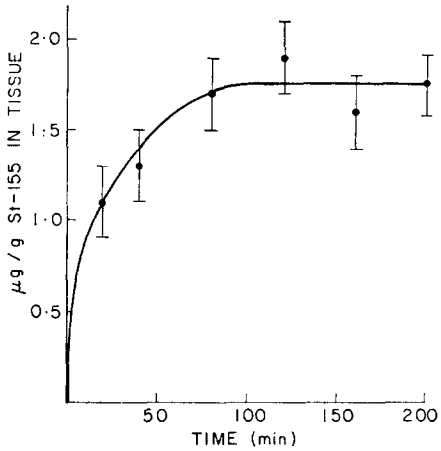

FIG. 6. Uptake of St-155 into submaxillary gland slices incubated in Krebs–Ringer solution containing the drug. (Starting concentration 0·14 μg/ml; see Table 4 for experimental details.)

TABLE 5. UPTAKE OF ST-155 INTO SLICES OF VARIOUS TISSUES

Tissue*	Concentration ratio
Submaxillary gland	15·3 ± 1·2 (3)
Stomach	3·6 ± 0·2 (3)
Heart	4·4 ± 0·5 (3)
Brain	4·7 ± 1·6 (2)
Muscle	6·7 ± 2·6 (2)

* Slices of rat tissues were incubated aerobically at 37° in Krebs–Ringer bicarbonate containing St-155 at a starting concentration of 0·5 μg/ml. After about 150 min, the compound reached a steady state distribution between the slice and the medium. The slice content: medium concentration ratio was determined at this point. The ratio are recorded ± S.E. with the number of experiments indicated in parentheses.

TABLE 6. UPTAKE OF ST-155 INTO SUBMAXILLARY GLAND SLICES AT VARIED pH*

Bath pH	Percentage of St-155 non-ionized in Ringer solution	Concentration ratio: St-155 in slices / St-155 in medium	Intracellular / Extracellular
5·5	0·8	1·3 ± 0·73	2·1
6·1	3·1	1·1 ± 0·41	1·6
6·4	5·9	3·0 ± 0·21	6·6
7·0	20·0	7·6 ± 0·15	18·6
7·4	38·7	12·1 ± 1·9	30·7
7·8	61·3	18·1 ± 3·3	46·2

* Slices of rat submaxillary gland were incubated aerobically at 37° in Krebs–Ringer containing St-155 at a starting concentration of 0·3 μg/ml. After equilibration had been reached, the slice: medium concentration ratio was determined. The last column shows the concentration ratio corrected for an extracellular (inulin) space value of 0·49 and total water of 0·87 ml/g tissue.

Experiments in which the partition of the radioactivity in the slices and Ringer solutions was measured between ethylene dichloride and buffer solutions at various pH values showed that no metabolism or decomposition of St-155 occurred in the tissue or bath fluids during the incubation.

DISCUSSION

Distribution of St-155 throughout those body compartments into which it penetrated was complete in the first 30 min after i.v. administration to rats, as shown by the exponential decline of the plasma concentration. The value for the apparent volume of distribution of St-155 indicated a high degree of localization of the compound in tissues and the similarity in half-lives of the drug in plasma and in tissues suggested that this localization was reversible.

The localization in submaxillary gland was particularly investigated because of its possible role in the salivary excretion of St-155. *In vivo*, the drug achieved an equilibrium distribution between the gland and plasma water of approximately 40 to 1, indicating 97·5 per cent localization of the drug in the tissue. The fact that this localization was chiefly in the gland and not in the saliva was demonstrated by the experiments with the cat preparation. The possibility that the localization was the result of reversible binding was investigated with tissue homogenates. However, reversible binding to a theoretical 100 per cent homogenate was estimated to be not more than 57 per cent corresponding to a 2 to 1 distribution between the gland and plasma water *in vivo*. Thus reversible binding was implicated only partially in the localization *in vivo*.

Experiments with submaxillary gland slices (S/M = 15·3) essentially reproduced the uptake *in vivo*. This uptake into slices was pH dependent (Table 6). A concept consistent with these results is that the intracellular pH of the salivary gland is considerably less than 7·4 and that the uptake resulted from passive diffusion of the non-ionized form of the compound across a membrane selective for lipid-soluble molecules. *In vivo*, such a membrane could occur at the interface between the gland and plasma water and also at the interface between the gland and saliva. Evidence for this was provided by the value of the concentration ratio of non-ionized St-155 in plasma water to that in saliva, which was close to unity. The pH differences would be eliminated with the destruction of cell membranes, such as occurs on homogenization and to some extent on slicing. Thus, the distribution of St-155 and probably of other drugs in saliva, submaxilllary gland and plasma water may occur by passive processes maintained in equilibrium by lipid membranes and pH differences. The gland apparently provides a "reservoir" for the slow excretion of weak electrolytes in saliva. These processes are being investigated further with St-155 and other compounds. It is noteworthy that the pH of human saliva is lower (5·17–6·71) than the pH of plasma.[5] If the above conclusions are correct, concentrations of basic compounds in human saliva can be expected to exceed the concentrations in plasma.

Specificity experiments indicated that metabolites of St-155 do not accumulate in submaxillary gland and are not excreted in saliva. The saliva results are in agreement with the concept that the salivary glands are most permeable to the non-ionized forms of weak electrolytes and to relatively lipid-soluble drugs.[6] The apparent selectivity of the reservoir for lipid-soluble molecules probably restricts salivary excretion of relatively water-soluble compounds.

The discovery that no accumulation of St-155 occurs in tissues without the presence of the material in plasma and that the half-life in plasma is similar to the half-life in the tissues examined indicates the validity of studies of plasma levels of the drug in animals and man as being representitive of the total body content and of levels at sites of action. Studies of binding to plasma proteins showed that, in the range under consideration, a near-constant proportion (about 55 per cent) of the St-155 in the plasma was bound to plasma proteins. Therefore, total plasma concentration is proportional to the concentration in the various body water compartments in diffusion equilibrium with plasma water. It should be possible to examine the relationship between plasma levels and the pharmacological[7] and toxic effects of the compound and to determine whether any intra- and inter-patient variations in response to St-155 in man and any interspecies differences in activity result from variations in rates of metabolism. Preliminary attempts with GLC to demonstrate the presence of St-155 in the plasma of patients under treatment have been unsuccessful owing to the very low dose (0·001–0·005 mg/kg) used to obtain hypotensive effects.[8]

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