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LOCALIZATION OF BASIC DRUGS IN THE SUBMAXILLARY GLAND

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Abstract—Numerous basic drugs have been reported to accumulate in the submaxillary gland. In this report an investigation of the nature of this uptake process is described using the basic drugs Ba 31531 {4-[5-amino-3-(4-pyridyl-1-pyrazolyl)-1-methylpiperidine] and ST-155 [2-(2,6-dichloroanilino)-2-imidazoline HCl]}. These compounds are reversibly taken up into the gland by a process with a high capacity which could be reproduced *in vitro* with tissue slices. The uptake into tissue slices exhibited a pH dependency which suggested that the accumulation was the result of a low intracellular pH. However, the intracellular pH determined with DMO was too high to account for this uptake. Attempts to account for the uptake by nonspecific binding to tissue components in homogenates also failed, suggesting that the uptake process requires an intact cell.

A NUMBER of studies have demonstrated that basic drugs, such as nicotine,[1] guanethidine,[2] and ST–155 [2-(2, 6-dichloroanilino)-2-imidazoline HCl],[3] a hypotensive agent, are extensively accumulated in the submaxillary gland. In addition, autoradiographic studies have shown a similar localization in the submaxillary gland of mepivocain, a local anesthetic,[4] and chlorpromazine.[5]

In the present studies the nature of the concentrating mechanism in the submaxillary gland has been investigated, using Ba 31531 {4-[5-amino-3-(4-pyridyl)-1-pyrazolyl]-1-methylpiperidine} (Ciba), a basic vasodilator drug,[6] and ST-155.[7] Both of these compounds were localized in the submaxillary gland of cats and rats at tissue to plasma ratios of approximately 20 to 1.[3,8] This report describes the results of a more extensive study of the physiological disposition of Ba 31531 and an examination of the role of pH in the localization of compounds in the submaxillary gland.

METHODS AND MATERIALS

Labeled compounds. DMO-2-^{14}C [5,5-dimethyloxazolidine-2,4-dione] (60 μc/mg, New England Nuclear Corp., Boston) was diluted with a nonlabeled drug to a specific activity of about 0·13 μc/mg. Ba 31531 {4-[5-amino-3-(4-pyridyl)-1-pyrazolyl]-1-methylpiperidine} (2 μc/mg, Ciba, Basel) was labeled in position 5 of the pyrazole ring. ST-155 [2-(2,6-dichloroanilino)-2-imidazoline HCl] (4·6 μc/mg, Boehringer, Ingleheim) was labeled in position 2 of the imidazoline ring. Inulin-carboxyl-^{14}C (3·6 μc/mg from New England Nuclear Corp.) was diluted with nonlabeled substance to a specific activity of about 0·25 μc/mg.

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Animal procedures. The animals used in this study were male Sprague–Dawley rats (180–250 g) and male cats (3–4 kg). Inulin space (Table 1) was determined from the volume of distribution of inulin-carboxyl-^{14}C in anesthetized rats (pentobarbital, 40 mg/kg i.p.) by ligation of the renal artery and vein. For tissue distribution studies, the rats were anesthetized with ether and blood was collected by cardiac puncture and added to about 0·1 ml of 0·05 % sodium citrate solution. In studies of drug concentration in saliva, cats were anesthetized with pentobarbital (40 mg/kg i.p.) and constant salivary flow was maintained by infusion of carbachol as described previously.[3]

Table 1. Tissue water, inulin space and DMO ratios*

Tissue	Tissue water (%)	Inulin space (%)	DMO ratio
Submaxillary gland	75 ± 0·5 (11)	21 ± 2·5 (10)	0·54 ± 0·04 (15)
Muscle (neck)	77 ± 0·1 (4)	17 ± 2·5 (5)	0·54 ± 0·04 (9)
Muscle (leg)	75 ± 0·15 (4)	12 ± 3·5 (5)	
Stomach	78 ± 0·6 (8)	25 ± 2·6 (10)	0·37 ± 0·01 (9)

*The animals were killed 1·5 hr after the administration of 25 mg/kg i.p. Values are means ± S.E. with the number of animals in brackets. The DMO ratio is the ratio of cpm/g wet tissue to cpm/ml plasma. Per cent refers to per cent of wet tissue weight.

Assay of various substances in tissues. For the assay of Ba 31531, tissues were homogenized in 9 vol. of 0·1 N HCl. A 1- or 2-ml aliquot of tissue homogenate was pipetted into a 50-ml glass-stoppered centrifuge tube containing 25 ml of chloroform and 1 ml of 5% NaOH. The tube was shaken for 10 min and centrifuged at 100 *g* for 10 min. The aqueous layer was removed by aspiration and the chloroform phase washed by shaking with 8 ml of 0·01 N NaOH. A 20- ml aliquot of the organic layer was transferred to a glass-stoppered centrifuge tube containing 1 ml of N HCl. The tube was shaken, centrifuged and a 0·5-ml sample of the aqueous layer was assayed by liquid scintillation spectrometry. Standards consisting of known amounts of BA 31531 were added to tissue homogenates and carried through the entire procedure. The recoveries found were: submaxillary gland, 80 per cent; heart, 77 per cent; muscle, 73 per cent; plasma, 85 per cent; brain, 70 per cent; lung, 80 per cent; duodenum, 84 per cent; liver, 76 per cent; stomach, 88 per cent. The experimental tissue levels were corrected by multiplying each value by 100 per cent recovery. The specificity of the method was established by the technique of pH-partition ratios. As shown in Table 2, the partition ratios of authentic Ba 31531 and apparent Ba 31531 extracted from urine and liver were almost identical.

ST-155 was assayed as previously reported.[3] DMO and inulin were assayed by the procedures of Addanki *et al.*[9] Tissue water was determined from the loss of weight after drying for 48 hr at 100° *in vacuo*. Submaxillary gland slices were prepared and incubated[3] in Krebs–Ringer solution containing Tris, phosphate, or bicarbonate buffers and gassed with pure oxygen or oxygen–carbon dioxide (95/5) in the Krebs–Ringer bicarbonate. The pH was adjusted prior to incubation and checked at the end. The incubations were carried out at 37° for 120 min.

Tissue binding. Binding of drugs to nondiffusible components of plasma or tissue

TABLE 2. DISTRIBUTION OF BA 31531 AND APPARENT BA 31531 EXTRACTED FROM URINE BETWEEN CHLOROFORM AND AQUEOUS SOLUTIONS AT VARIOUS PH VALUES*

	Per cent	
pH	Apparent Ba 31531 urine	Authentic Ba 31531
6·4	97·0	98·5
7·6	63·5	64·0
8·0	51·5	55·0
8·8	30·5	31·5
9·0	29·0	29·5
10·2	22·0	23·5

*The apparent Ba 31531 was extracted from the urine of rats asd escribed under Methods. Aliquots of the extract and of an aqueous solution of Ba 31531 were adjusted to various pH values and shaken with chloroform. The amount of Ba 31531 remaining in the aqueous layer was determined and expressed as a percentage of the total material originally present.

homogenates was determined by equilibrium dialysis or by ultrafiltration as previously described.[3]

Apparent p*K*a values were determined by titration in 0·9% NaCl as described by Albert and Sargent.[10]

RESULTS

The concentration of Ba 31531 was measured in various tissues 1 hr after the intravenous injection of 1, 5, and 100 mg/kg of the drug (Table 3). Only in the liver was the concentration of drug as high as in the submaxillary gland. The concentration of drug in the latter tissue was about twenty times that in plasma over a 100-fold range in dosage, suggesting that the process of drug accumulation had not been saturated. The time course of the Ba 31531 content in a number of tissues showed that the tissue

TABLE 3. DISTRIBUTION OF Ba 31531 IN RAT TISSUE AFTER THE ADMINISTRATION OF VARIOUS DOSES OF THE DRUG

Tissue	Dose of Ba 31531		
	1 μg/kg, i.v. (μg/g)	5 μg/kg, i.v. (μg/g)	10 μg/kg, i.v. (μg/g)
Submaxillary gland	2·8 (2·7–3·0)*	13·5 (11·1–15·3)*	338 (232–456)*
Heart	0·4 (0·2–0·5)	2·3 (1·9–3·0)	47 (41–67)
Muscle	0·6 (0·6–0·7)	2·2 (1·9–2·6)	52 (37–87)
Plasma	0·2 (0·1–0·3)	0·7 (0·5–1·0)	17 (11–23)
Brain	0·2 (0·1–0·3)	1·0 (0·6–1·3)	24 (14–35)
Lung	1·5 (0·6–2·4)	6·6 (6·0–7·5)	148 (112–178)
Duodenum	0·5 (0·4–0·6)	3·0 (2·9–3·1)	66 (38–99)
Liver	3·2 (2·9–3·5)	13·7 (13·1–14·3)	182 (97–254)
Stomach	1·1 (0·8–1·4)	6·0 (5·6–6·3)	93 (36–152)
No. animals	2	3	5

*Mean values (range).

and plasma concentrations of drug declined in a parallel manner (Table 4). Similar data have been reported for ST-155.[3]

Tissue to plasma ratios of DMO were determined together with inulin space and tissue water to calculate the intracellular pH[11] (Table 1). The values for leg muscle were in agreement with Addanki *et al.*[9] DMO, which is a weak acid, was also used to examine the behaviour of an acidic compound in the experimental procedures.

The average saliva levels in cats of the two bases and DMO over a 90-min period after intravenous injection are shown in Table 5. The saliva concentration of DMO was higher than the plasma concentration, while the reverse relationship was found for the bases. The levels of Ba 31531 and ST-155 in the gland were considerably higher than the plasma concentrations. In contrast, the concentration of DMO in the submaxillary gland was approximately the same as in plasma and saliva.

TABLE 4. CONCENTRATION OF Ba 31531 IN RAT TISSUES AT VARIOUS TIMES AFTER ADMINISTRATION OF DRUG (5 mg/kg, i.v.)*

Tissue	Time				
	0·5 hr (μg/g)	1 hr (μg/g)	2 hr (μg/g)	4 hr (μg/g)	8 hr (μg/g)
Submaxillary gland	17·0 (1)	12·7 (9·5–15·3) (5)	7·7 (5·5–10·8) (3)	3·5 (3·2–3·8) (3)	2·6 (2·5–2·7) (2)
Stomach	5·7 (1)	6·0 (5·6–6·3) (3)	3·7 (3·5–4·0) (2)	3·8 (1)	1·5 (1·4–1·6) (2)
Heart	2·9 (1)	2·3 (1·9–3·0) (5)	1·7 (1·4–1·9) (3)	0·5 (0·3–0·6) (3)	0·4 (0·5–0·4) (2)
Muscle	2·6 (1)	2·2 (1·9–2·6) (5)	1·4 (1·2–1·6) (4)	0·7 (0·5–0·9) (4)	0·3 (0·3–0·4) (2)
Plasma	0·9 (1)	0·6 (0·5–1·0) (5)	0·4 (0·4) (3)	0·15 (0·1–0·2) (4)	0·1 (0·1) (2)

*Mean values with the range in brackets. The single numbers in brackets refer to the number of animals.

TABLE 5. CONCENTRATION OF WEAK ORGANIC ELECTROLYTES IN THE PLASMA, SALIVA, AND SUBMAXILLARY GLAND OF THE CAT*

Compound	Dose (i.v.)	Plasma concentration (μg/ml)	Saliva concentration (μg/ml)	Submaxillary gland (μg/g)
Ba 31531	5 mg/kg	2·1	0·91	29·5
DMO	20 mg/kg	35·1	41·1	24
ST-155	250 μg/kg	0·04	0·0013	1·4

*The plasma and saliva levels are the mean of 4 samples collected over a 90-min period while the gland levels represent the average of the 2 glands taken 90 min after injection. The pH of the saliva, collected in a syringe to prevent loss of CO_2, was 7·65.

DMO, Ba 31531 and ST-155 were all taken up by slices of submaxillary gland incubated in Krebs–Ringer solution reaching a steady-state equilibrium within 90 min. The role of pH in this uptake was examined by varying the medium pH. The uptake of the two basic compounds increased from a slice to medium ratio of about 1 at a medium pH of 6 to 18 at pH 7·8. A similar pH dependency has been reported for the uptake of nicotine by submaxillary slices.[1] The uptake of the weakly acidic DMO exhibited a slight decrease with increasing medium pH, and the slice to medium ratio was always less than 1 (Table 6).

TABLE 6. EFFECT OF MEDIUM pH ON UPTAKE OF WEAK ELECTROLYTES BY RAT SUBMAXILLARY SLICES

Compound	Medium concentration (μg/ml)	Medium pH	Slice concentration (μg/g)* Medium concentration (μg/ml)
Ba 31531	0·5	6·2	1·1 (0·9–1·8)
		7·1	3·7 (3·1–4·3)
		7·6	12·0 (10·8–12·7
		7·7	13·5 (12·7–15·1)
DMO	0·17	5·9	0·88 (0·82–0·94)
		6·7	0·85 (0·75–0·92)
		7·0	0·79 (0·72–0·84)
		7·3	0·72 (0·67–0·77)
		7·6	0·67 (0·62–0·75)
ST–155	0·5	6·1	1·1 (0·8–1·5)
		7·0	7·6 (6·1–9·0)
		7·4	12·1 (10·2–14)
		7·8	18·1 (15 –21·7)

*Mean of three values (range).

The binding of Ba 31531 to constituents of 10 per cent tissue homogenates was 27 per cent for submaxillary gland, 10 per cent for heart and 9 per cent for skeletal muscle. The binding of this compound to constituents of whole plasma was 22 per cent. A comparison of the effect of pH on binding to submaxillary gland homogenates is shown in Table 7. Unlike the uptake by slices, the binding to homogenates by the bases was only slightly affected by pH. DMO did not bind to homogenates under these conditions.

TABLE 7. EFFECT OF pH ON BINDING TO RAT SUBMAXILLARY GLAND HOMOGENATES

pH	ST–155 (% bound)*	Ba 31531 (% bound)*
5·8	31·0	55·0
6·5	47·0	44·0
7·0	57·0	33·3

*Values are extrapolated[12] to a theoretical 100 per cent homogenate based on binding data for 12·5, 25 and 50 per cent tissue homogenates prepared as described in Methods. DMO did not bind under these conditions. The concentration of the drugs was 1·2 μg/ml for ST–155 and 3 μg/ml for Ba 31531.

DISCUSSION

The data presented on the distribution of Ba 31531 in various tissues show that it is extensively localized in the submaxillary gland. The parallel decline in tissue and plasma levels indicates that the uptake is reversible, and the near constant tissue to plasma ratio ranging over two orders of magnitude indicates the uptake system is one with a high capacity. As reported previously, ST-155 gives similar results in analogous experiments. The localization of both basic drugs occurs within the gland and not the saliva as shown by the data of Table 5. In contrast, the acidic DMO did

not exhibit this accumulation. The variation in chemical structure and pharmacological action of these and other basic compounds reported to accumulate in the submaxillary gland suggested a general mechanism dependent on physical properties may be involved in the uptake process.

Additional support for this possibility was provided by the studies *in vitro* where the effect of medium pH on uptake was examined. The increase in uptake of the basic compounds with increasing medium pH and the lower uptake of the weak acid suggested that the accumulation of bases in the submaxillary gland could be the result of a low intracellular pH. The uptake was then examined in terms of a pH-partition system consisting of aqueous compartments (intracellular and extracellular water) of differing pH separated by a lipid membrane penetrable only by the unionized form of a weak electrolyte.[13] The intracellular pH required for the high submaxillary gland/plasma concentration ratio observed is about 6. However, the intracellular pH, as determined by DMO partition, is about 7·0, the same as that of muscle (see Table 8). This pH value would predict equal muscle and submaxillary gland levels for the basic compounds.

Table 8. Intracellular water/plasma water concentration ratios

Tissue	pH_i*	Intracellular concentration/Plasma concentration			
		Ba 31531		ST-155	
		Found†	Calc.‡	Found	Calc.
Submaxillary gland	7·17	30·6	1·62	41·3	1·31
Muscle	7·16	5·0	1·66	7·05	1·31
Stomach	6·65	5·15	5·15	11·0	3·47
p*K*a		8·3		7·6	

*Intracellular pH (pH_i) was determined from the data of Table 1 and the equation of Waddell and Butler.[11]

†Concentration ratio of unbound drug/free plasma level after correction for binding. The equation used[14] is:

$$\frac{C_i}{C_m} = \left[\frac{C_t}{C_m} \cdot \frac{1}{(1 - S_s/S_w)} - \frac{S_s/S_w}{(1 - S_s/S_w)}\right] \cdot \left(1 - \frac{\%\ \text{bound}}{100}\right)$$

Where C_i = concentration in intracellular water, C_m = concentration in extracellular water, S_s = inulin space, and S_w = total water, C_t = concentration in tissue water.

‡Calculated from the equation:[13]

$$\frac{C_i}{C_m} = \frac{1 + 10^{pKa - pH_i}}{1 + 10^{pKa - 7{\cdot}4}}$$

where pH_i is determined with DMO.

The deviation of the observed tissue uptake from the uptake predicted by the pH partition and the determined intracellular pH is shown in Table 8. The tissue levels were converted to the concentration of unbound drug in intracellular water by correction for binding at pH 7·0 and inulin space. The ratio of the free concentration in intracellular water to plasma water calculated from the DMO derived intracellular pH is close to 1·5 for muscle and submaxillary gland and 4 to 5 for stomach. The wide discrepancies between the found and calculated ratios indicate that pH partition alone

cannot account for the accumulation of bases by the submaxillary gland with an intracellular pH of 7·17.

An error in the measurement of intracellular pH by DMO could result if the compound were bound to a tissue component or had accumulated in the saliva. However, there is no evidence of DMO binding at pH 5·8, 6·5, or 7·0 under the conditions described in Table 6, and the saliva levels in the anesthetized cat (Table 5) are comparable to plasma levels. In fact, saliva pH values calculated from saliva/plasma ratios of both Ba 31531 and DMO (7·74 and 7·47) are in reasonable agreement with the pH determined with pH meter (7·74), indicating that the saliva levels are consistent with a simple pH partition between plasma and saliva.

Alternatively, the high uptake, beyond that predicted by the pH–partition system, could reflect extensive tissue binding by bases. This possibility was examined with tissue homogenates and neither the extensive binding *in vivo* (a tissue plasma ratio 20:1 represents 95 per cent binding) nor the pH dependency could be reproduced. Furthermore, the binding to tissue homogenates of different tissues did not reflect the differences in uptake by the intact tissue. For example, the binding of Ba 31531 to homogenates of submaxillary gland was 26 per cent and that for muscle was 10 per cent while a 20-fold difference was observed for uptake by intact cells. Hence, the interactions of the bases with tissue homogenates do not reflect the uptake by intact cells. However, it is possible that the binding in intact cells cannot be reproduced in homogenates. While homogenization liberates cellular constituents, the components are in an altered ionic environment. The ionic environment within a cell is controlled by energy requiring processes which are lost with the disruption of the cell. One type of binding that would be extremely sensitive to ionic environment is the Donnan-type attraction between the positively charged bases and negatively charged polyelectrolytes within the cell, described by Danielli.[15] It is possible that interactions of this type are very important in the uptake of bases by the submaxillary glands.

Thus, while weak organic bases are extensively accumulated by the submaxillary gland in a process that is readily reversible and nonsaturable, it is not accountable by a simple pH partition. While attempts were made to examine the contribution of binding by use of homogenates, the binding observed was not related to the uptake of the intact cell. While the mechanism is unknown, the uptake of bases by the submaxillary gland appears to be a general process and should be anticipated in studies of the physiological disposition of new drugs.

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