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Relationship between the uptake, binding and pharmacological action of procaine in the isolated heart

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1. The uptake, efflux and pharmacological actions of procaine hydrochloride were studied on isolated hearts of female guinea-pigs. The hearts were perfused with Krebs solution containing ^{14}C-procaine (0.1–500 μg/ml.) by the Langendorff technique at 37° C, using a constant flow pump. Hearts and cardiac effluent were assayed for procaine by liquid scintillation spectrometry.

2. Accumulation of procaine did not appear to involve active transport mechanisms for the following reasons. The rate of procaine uptake was most rapid at the highest perfusion concentration (500 μg/ml.), when it was three times faster than at the lowest concentration (0.1 μg/ml.); it was not affected by lowering the temperature to 3° C. The ratio of the concentration of procaine in the heart to the concentration in the perfusing fluid decreased with increasing concentration in the perfusion fluid.

3. When the efflux of ^{14}C-procaine from hearts previously perfused with procaine-containing Krebs solution for 10 min was compared with the efflux of ^{14}C-inulin, the patterns of efflux of both compounds were similar, and showed at least two exponential components. At the highest concentration of procaine (500 μg/ml.) the efflux of procaine was more rapid than that of inulin.

4. A relationship was found between the pharmacological action of procaine, the rate of uptake and the level of procaine in the heart. When the procaine-containing perfusion fluid was changed to a procaine-free solution, the heart rate increased rapidly, and there was a rapid decline in the levels of procaine in the hearts.

5. It is concluded that guinea-pig hearts accumulate procaine by a passive diffusion process, and that the pattern and rate of efflux indicate that the drug is loosely bound. If it is permissible to extrapolate from these findings to the antiarrhythmic effect in man, the short duration of its action may be due to loose binding rather than to rapid metabolic inactivation.

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The weak and transient antiarrhythmic actions of procaine on the heart are commonly considered to be due to its rapid metabolism to diethylaminoethanol and *p*-aminobenzoic acid (Brodie, Lief & Poet, 1948; Brodie, 1964). Although rapid metabolism may account for the short lasting effects of procaine, however, it cannot alone satisfactorily explain the drug's low potency. Because *p*-aminobenzoic acid is inactive as an antiarrhythmic agent and diethylaminoethanol has considerably less activity than the parent compound (Rosenberg, Kayden, Lief, Mark, Steele & Brodie, 1949), it seems likely that the cardiac effects may be mediated by procaine itself rather than by any metabolite, and that the low potency and short duration of action are due to the binding characteristics of the drug.

There is little information on the distribution of procaine in tissues, but there is evidence that the binding of procaine in squid axoplasm (Truant & Takman, 1965) and also in heart homogenates (Truant & Takman, 1959) is limited. Accordingly, we have investigated the uptake and binding of procaine in the isolated perfused heart of the female guinea-pig, and attempted to relate these parameters to the rate of onset and duration of pharmacological action. A preliminary account of some of these experiments has been given elsewhere (Boullin & Sullivan, 1967).

Methods

Hearts from virgin female guinea-pigs (300–400 g) were removed and perfused with Krebs solution by the Langendorff technique at a constant flow rate of 6 ± 0.4 ml./min by means of a peristaltic pump (model 600–1200, Harvard Apparatus Co., Boston).

In experiments designed to study the uptake and binding of procaine, after a preliminary perfusion with drug-free medium for 5 min, the solution was changed to one containing ^{14}C-procaine in concentrations of 0.1, 1.0, 100, 250, 350 or 500 μg/ml. When high concentrations of procaine 100–500 μg/ml. were used, the radioactive compound (1 or 10 μg/ml.) was diluted with non-radioactive procaine. The drug was perfused for 1–20 min, and 1 min samples of the cardiac effluent were collected throughout. Thereafter, in some experiments, perfusion was continued with drug-free Krebs solution for a further 10–15 min. At the end of the experiments the hearts and perfusates were assayed for radioactivity. The hearts were homogenized in 5 volumes of 0.4 N perchloric acid in an Ultra-Turrax homogenizer and allowed to stand for about 2 hr. At the end of this time the homogenates were centrifuged (600 *g*) for 5 min and 1 ml. of the clear supernatant was assayed for total radioactivity by liquid scintillation spectrometry (Boullin, 1966).

To identify the isotopes in hearts after administration of procaine, potassium hydroxide was added to perchloric extracts to precipitate perchlorate which was separated by centrifugation. The clear supernatant was bubbled with nitrogen overnight to reduce the volume and then applied to Whatman No. 1 paper. Ascending radiochromatograms of tissue extract and authentic procaine were run in methanol : acetone : triethanolamine (100 : 100 : 3 v/v/v). Authentic procaine and tissue extracts gave a single peak with an R_F value of 0.59. Of the radioactivity extracted from tissues, 94% was confined to within the peak.

To measure the extracellular space, hearts were perfused with ^{14}C-inulin and treated as above. Tissue levels of procaine (C_i) are expressed as μg/g after deducting the content of the extracellular (inulin) space which was 0.44 ml./g. The tissue/

perfusion medium ratio is given by C_i/C_o where C_o is the concentration in the medium.

In experiments designed to study the pharmacological effects of procaine, hearts perfused by the Langendorff technique were attached to a force-displacement transducer (Grass, Model FT.O3C), the output voltage from which was displayed on a multichannel polygraph (Devices, M.4). Heart rate (beats/min) and amplitude of contractions (mm) were measured from the tracings obtained during administration of procaine (100, 250, 350, and 500 μg/ml.) for up to 20 min.

Drugs

^{14}C-carboxyl procaine hydrochloride (specific activity 8.33 μc/mg) and ^{14}C-carboxyl inulin (specific activity 3.08 μc/mg) were purchased from New England Nuclear Corporation, Boston. Concentrations of procaine refer to the weight of salt.

Results

Preliminary experiments showed that concentrations of 0.1 and 1.0 μg/ml. of procaine had no pharmacological action on the isolated perfused heart, whereas concentrations of 100, 250, 350 and 500 μg/ml. had pronounced negative chronotropic and inotropic effects. The highest concentration studied was 500 μg/ml. because this concentration invariably caused ventricular arrest. With 250 and 350 μg/ml. ventricular arrest occurred in a proportion of hearts, but with 100 μg/ml. it never occurred.

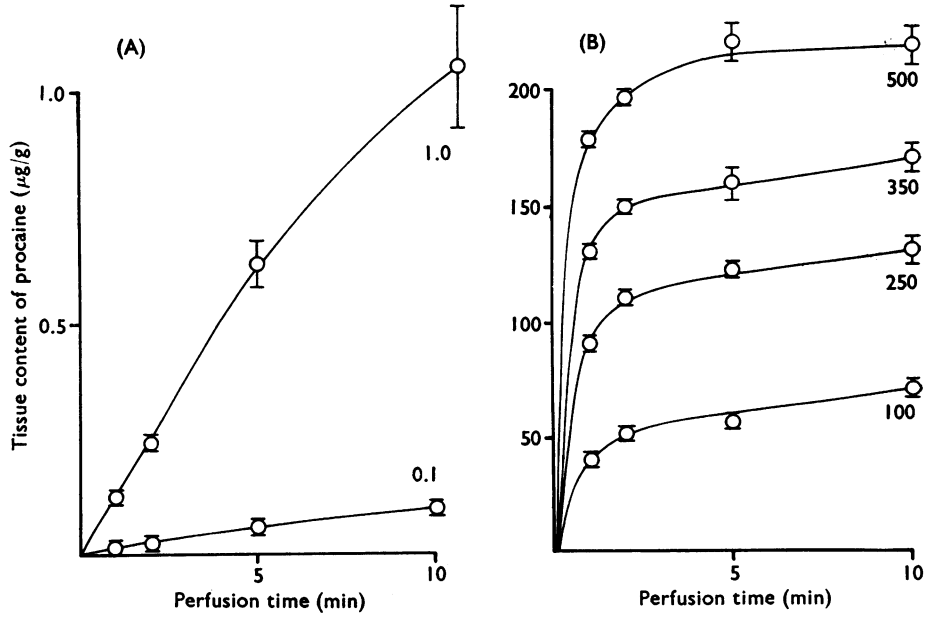

FIG. 1. ^{14}C-procaine content of guinea-pig isolated hearts plotted against time (min) of exposure to procaine. Each point is the mean of four or five experiments, the vertical bars indicating ±S.E. Procaine concentrations: (A) 0.1 and 1 μg/ml.; (B) 100, 250, 350 and 500 μg/ml.

Uptake of procaine

The pattern of uptake of procaine by hearts perfused for up to 10 min is shown in Fig. 1 and Table 1. It will be seen that the higher perfusion concentrations produced the greatest initial rates of uptake and that steady state conditions were therefore approached more quickly. They were, in fact, attained when 500 μg/ml. of procaine was administered for 10 min. The C_i/C_o ratios after exposure to procaine for 10 min were found to be inversely proportional to the concentrations of procaine (Table 2), and were considerably lower than might have been expected had accumulation involved only a simple diffusional process. The ratio was as low as 0.44 with 500 μg/ml., and even the highest ratio, obtained with 1 μg/ml., was just above unity.

The effect of lowering the temperature on uptake was studied. When six hearts were perfused for 10 min with procaine in concentrations of 1 or 500 μg/ml. at 3° C, uptake was no less than that at 37° C (Table 3). A decrease in the uptake of procaine would have been expected at 3° C if accumulation involved metabolically dependent mechanisms. This observation, and the other results given above, are compatible with the view that procaine uptake was by diffusion rather than by

TABLE 1. *Effect of the duration of procaine administration on the rate of uptake by the isolated perfused heart*

Procaine in perfusion fluid (μg/ml.)	Mean rate of uptake of procaine (μg/g per min ± S.E.)			
	0–1	1–2	2–5	5–10
0·1	0·012±0·001 (2·1)	0·012±0·001 (2·1)	0·008±0·001 (1·33)	0·008±0·001 (1·33)
1	0·12±0·006 (2·1)	0·12±0·008 (2·1)	0·09±0·01 (1·43)	0·09±0·01 (1·43)
100	41·2±1·24 (6·9)	10·2±0·90 (1·7)	1·5±0·09 (0·25)	2·8±0·13 (0·47)
250	90·0±2·5 (6·0)	21·0±1·96 (1·4)	4·0±0·38 (0·28)	1·4±0·11 (0·1)
350	130±3·9 (6·2)	19·1±2·0 (0·9)	3·3±0·17 (0·15)	2·2±0·2 (0·1)
500	179±2·9 (6·0)	17·0±1·61 (0·6)	8·7±1·12 (0·29)	NIL

Hearts were perfused at a rate of 6 ml./min with Krebs solution containing procaine 0·1, 1, 100, 250, 350 and 500 μg/ml. for 1 to 10 min. The rates of uptake were calculated from the data shown in Figs. 1 and 2; the values are the means of four or five experiments. The percentage of the administered dose that was taken up in successive periods is shown in parentheses.

TABLE 2. *Relationship between increasing perfusion concentration of ^{14}C-procaine and C_i/C_o ratios*

Perfusion concentration (μg/ml.)	Ratio C_i/C_o
0·1	1·01
1	1·05
100	0·70
250	0·52
350	0·48
500	0·44

C_i is procaine uptake by the heart after correction for the extracellular space, and C_o is the concentration in the medium. Results given were obtained after perfusion for 10 min.

energy dependent mechanisms and that the low C_i/C_o ratios could perhaps be associated with the pattern of procaine release after uptake.

Efflux of procaine

The efflux of procaine from perfused hearts was investigated after perfusion with various concentrations, and was compared with the efflux of inulin (Fig. 2). For both substances, the efflux curves consist of at least two exponential components: an initial rapid phase with time constant ($t\frac{1}{2}$) of 0.4–0.8 min, followed by a slower phase with time constants in the range 1–3 min (Table 4). The time constants of the first and second phases of procaine efflux show decreases with increasing con-

TABLE 3. *Effect of temperature on uptake of ^{14}C-procaine by isolated hearts*

Procaine in perfusion fluid (μg/ml.)	Uptake (μg/g)	
	3° C	37° C
1	1·03±0·09	1·05±0·13
500	216±9·1	222±8·3

Procaine was administered for 10 min. Each value is the mean (±S.E.) of three to five determinations.

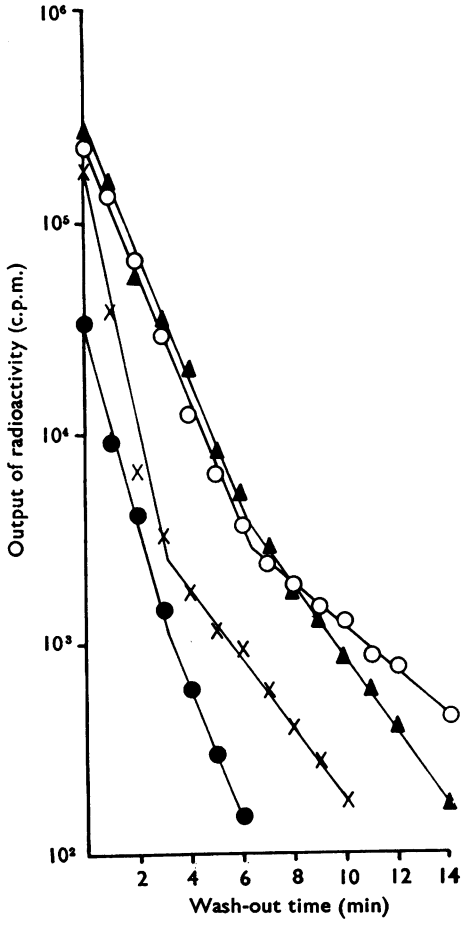

FIG. 2.—Efflux of procaine after perfusion of hearts with three concentrations of procaine (○——○, 1 μg/ml.; ▲——▲, 100 μg/ml.; ●——●, 500 μg/ml.) compared with the efflux of inulin (×——×, 1 μg/ml.). Hearts were first perfused with drug solutions for 10 min and then with drug-free Krebs solution (wash-out), and the radioactivity of the effluent determined. Radioactivity (c.p.m.) is plotted against wash-out time (min).

centrations, so that the most rapid efflux was seen with 500 μg/ml.; at this concentration, the rate of procaine efflux during the second phase exceeded that of inulin, which served as marker for the effluent from the extracellular space.

These results suggest that the small C_i/C_o ratios found when hearts were perfused with high concentrations of procaine, may have been due not only to saturation of the binding sites, but also to the extremely rapid rate of procaine efflux.

Pharmacological effects of procaine

The inhibitory actions of procaine were studied only on the ventricles, because the atria were relatively resistant to the drug. When concentrations of procaine sufficient to cause ventricular arrest were used, the atria either stopped beating only at a later stage or continued to beat at a reduced rate throughout perfusions lasting for 20 min.

TABLE 4. *Time constants of efflux of procaine and inulin from isolated perfused hearts*

Drug	Perfusion concentration (μg/ml.)	Time constant (min) (*a*)	(*b*)	Number of observations
Inulin	1	0·4	1·7	4
Procaine	1	0·8	3·0	4
	100	0·8	1·7	5
	500	0·5	1·0	9

Results refer to the time constants ($t_{\frac{1}{2}}$) of the first (*a*) and second (*b*) components of the exponential efflux curves shown in Fig. 2.

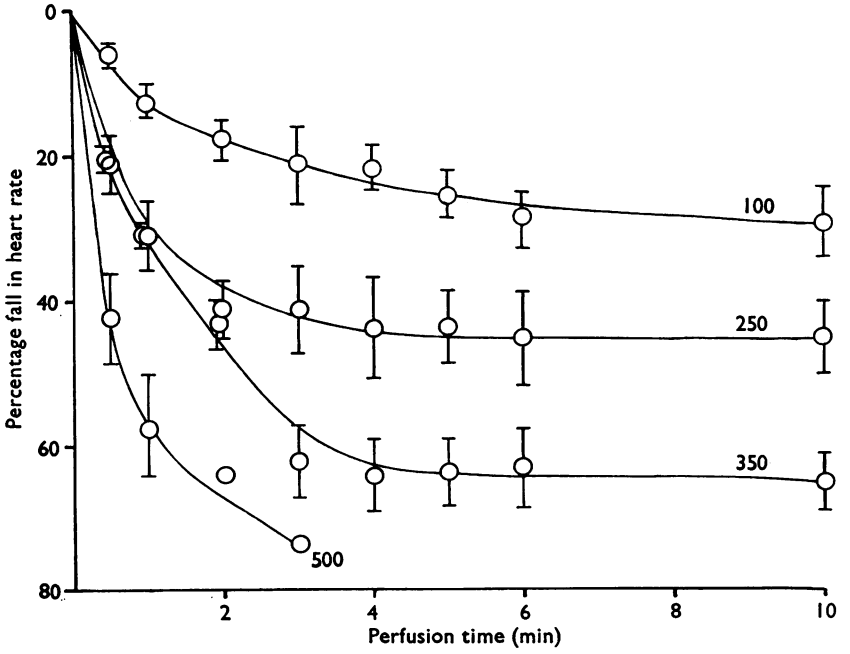

FIG. 3. Decrease in heart rate after perfusion with different concentrations of procaine. Percentage fall in heart rate is plotted against time (min) of exposure to procaine. The figures at the end of each curve indicate concentrations of procaine (μg/ml.). Each point is the mean of three to twelve experiments ±S.E., with the exception of the last two points of the curve for 500 μg/ml. (two experiments).

Figure 3 shows the negative chronotropic effects of procaine administered for 10 min. With all pharmacologically active concentrations (100–500 μg/ml.), the onset of the effect developed within 10 sec of procaine reaching the heart. There was a rapid decline in the heart rate during the first few minutes, a period which coincided with the most rapid rate of accumulation of procaine (Fig. 1); but after 5 min there was no further decrease in the heart rate. Ventricular arrest developed very quickly when the hearts were perfused with 500 μg/ml. of procaine.

The negative inotropic effect followed a similar pattern, except that during the first minute of exposure the fall in amplitude of contraction was often much more marked than the decrease in rate, and was then followed by a partial recovery. Thereafter, the negative inotropic and chronotropic effects were of approximately the same order of magnitude.

Relationship between procaine uptake and pharmacological action

It has been shown that during the first few minutes of procaine administration the rate of procaine uptake is greatest (Table 1), most of the procaine finally found in

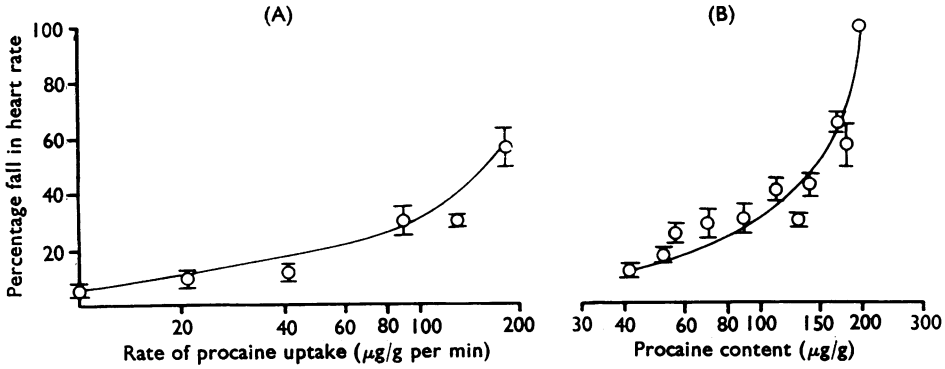

FIG. 4. Relationship between percentage reduction in the heart rate and (A) rate of procaine uptake (μg/g per min), and (B) procaine content (μg/g). Each point is the mean of four to ten experiments ±S.E. Results in (A) were obtained by measuring the heart rate and estimating the content of procaine in hearts at the end of 1 min administration of procaine (100–500 μg/ml.). Results in (B) were obtained by measuring the heart rate and estimating the procaine content in hearts at different times after the start of administration of procaine (100–500 μg/ml.).

TABLE 5. *Relationship between procaine concentration and cardiac arrest in isolated hearts*

Procaine in perfusion fluid (μg/ml.)	Time to ventricular arrest (sec)	Time to tissue assay (min)	Procaine content of the hearts (μg/g)
100	—	10	70±4·2
250	—	10	130±4·8
350	—	1	130±3·9
	—	2	149,150
	115	2	156,161,168
500	—	1	179±2·9
	110–120	2	196±4·1
500	110–120	1*	117±2·4

Hearts were perfused with Krebs solution containing procaine for up to 10 min. The procaine contents are the means of three to five experiments ± S.E.
* The perfusion of four hearts with procaine-Krebs solution was continued for 10 min; the procaine content was estimated after a further 1 min perfusion with procaine-free medium.

the hearts has already accumulated (Fig. 1) and the development of the pharmacological action is most rapid (Fig. 3). The relationships between the rate of procaine uptake and procaine content of hearts on the one hand, and the negative chronotropic effect on the other, are shown in Fig. 4. The dose-response curve is not of the familiar sigmoid shape, a finding which will be considered in detail in the Discussion.

The results of experiments carried out to study the relationship between procaine levels in the heart and maximum pharmacological effect as determined by the occurrence of ventricular arrest are shown in Table 5. Hearts were perfused for up to 10 min until ventricular arrest occurred. They were then removed within 10 sec for estimation of the procaine content. The results show that procaine concentrations ranging from 156 to 196 μg/g were necessary to cause ventricular arrest. On the other hand, ventricular arrest did not develop in the hearts perfused with solutions containing 100 and 250 μg/ml. of procaine for 10 min, and in these experiments the tissue levels did not rise above 130 μg/g. In three out of ten hearts treated with procaine 350 μg/ml., ventricular arrest occurred in 115 sec. The mean level of procaine in the three hearts which stopped beating was greater than 150 μg/g, whereas in the other seven hearts it was 150 μg/g or less. There was some variability in the sensitivity of hearts to procaine, however, because when a concentration of 500 μg/ml. was used, five hearts were still beating when the mean tissue level had reached 179 μg/g.

Finally, some experiments were carried out to study the relationship between recovery from the pharmacological effects of procaine and wash-out of procaine from the heart. Four hearts were perfused with procaine 500 μg/ml. for a full 10 min although ventricular arrest had occurred about 2 min after the start of procaine administration. At the end of the 10 min period, the hearts were perfused with procaine-free Krebs solution for 1 min. The recovery of cardiac activity was very rapid, the atria beginning to beat in a mean time of 17.5 sec, the ventricles in 32.5 sec. Estimation of the procaine content of these hearts at the end of the 1 min wash-out period showed that the mean content was 117 μg/g (Table 5), which was well below the level associated with ventricular arrest in other experiments.

The extreme rapidity with which hearts began beating after washing out procaine did not seem to be related to the duration of the perfusion with procaine. Two hearts were perfused with a solution containing procaine 500 μg/ml. When ventricular arrest had occurred, in 38 and 190 sec respectively, the hearts were at once perfused with drug-free Krebs solution. The ventricles began to beat again in 38 and 18 sec. Perfusion with the procaine-containing solution was then repeated, and was continued for 20 min irrespective of the development of ventricular arrest. When the hearts were then perfused once more with drug-free Krebs solution, the ventricles began to beat in 85 and 20 sec.

Discussion

The experiments we have described suggest that there is a relationship between the overall accumulation of procaine by the heart and its pharmacological effect. In addition we have obtained information about the accumulation of procaine and the degree of binding.

Our results indicate that uptake of procaine is by a process of passive diffusion rather than by an energy dependent mechanism. For example, the rate of uptake was most rapid with the highest perfusion concentrations, and the amount taken up was no less at 3° C than at 37° C. Solomon & Zieve (1967) considered that the uptake of procaine by human blood platelets was also by a process of diffusion. They found that steady state conditions were attained after 5 min incubation with procaine (100 μg/ml.) when the C_i/C_o ratio was 2.85. In our experiments, C_i/C_o ratios were never greater than 1.05 after 10 min perfusion, and furthermore they were inversely related to the procaine concentrations; the lowest ratio, 0.44, was obtained with a concentration of 500 μg/ml. If uptake is dependent on diffusion alone, at equilibrium a value of C_i/C_o of unity is to be expected; when intracellular binding occurs or transport is metabolically dependent, higher ratios are commonly found.

The degree of ionization may have influenced the amount of procaine binding, for it is believed that only the unionized form of a local anaesthetic molecule penetrates nervous tissues (Ritchie & Greengard, 1966). If this holds for the heart also, then the " effective " concentrations of procaine in our experiments may have been as low as 1.7% of the actual concentration, because at pH 7.2 procaine is 98.3% ionized (Bianchi & Bolton, 1967). Another influence on the cellular binding of procaine may be the degree of binding to proteins. Solomon & Zieve (1967), however, found that whereas protein binding played a part in the uptake of procaine by blood platelets at pH 8, this was not so at pH 7.4.

The efflux curves for all concentrations of procaine consisted of at least two exponential components and the rate of procaine loss became faster with increasing concentrations of procaine. At concentrations of 500 μg/ml., the rate of efflux of procaine during the second, slower, phase of the efflux curve was actually greater that that of the extracellular marker, inulin. Recently, Young (1968) using isolated perfused rat hearts, has described similar biphasic efflux curves during wash-out of extracellular markers such as inulin, raffinose, sorbitol and sucrose, and of substances which penetrate the interstitial space, such as Evans blue-albumin conjugate. The pattern and time constants of efflux found for inulin by Young (1968) are similar to those found for procaine in the present experiments. Consequently it is pertinent to the present work that he reported that the fast phase of efflux results from heart contractions and is absent in the quiescent organ. Since Young (1968) has shown that isolation of the rat heart and its perfusion *in vitro* with Krebs solution causes changes in capillary permeability which affect the flow rate, and alter the pattern of flow between the capillaries, arterioles and venules, it may be concluded that the biphasic efflux of procaine from the guinea-pig heart represents loss of substance from vascular and extracellular spaces, and that the increased efflux that occurs when the procaine concentration in the perfusing fluid is raised represents additional loss of drug from binding sites.

There is no simple relationship between the tissue levels of procaine and its pharmacological effect. Both the rate of uptake of procaine and the tissue content are related to its negative chronotrophic effect. The dose-response curve has not a sigmoid shape, because the procaine level at the time of cardiac arrest (=100% response) is lower than the predicted value. This is probably due to the fact that, with increasing procaine content, the ventricular rate does not gradually fall to zero, as is shown by the following observation. When eleven hearts were perfused

with procaine until ventricular arrest occurred, the last mean recorded rate immediately before arrest was $40.4 \pm 4.5\%$ of the control rate; that is, once the heart rate had fallen to this level, ventricular arrest then followed abruptly. Conversely, when the ventricular rate was reduced to 60% of the control rate the procaine content was only slightly less than at ventricular arrest.

There is a remarkable similarity between the uptake of procaine described here and the accumulation of tritiated ouabain by guinea-pig atria described by Kuschinsky, Lüllman & Van Zwieten (1968). With both procaine and ouabain C_i/C_o ratios were less than unity, steady state conditions were not attained for 20 min and efflux was rapid. This contrasts with the results obtained by Kuschinsky *et al.* (1968) for digitoxin, which was concentrated to a much greater degree. These authors suggest that only a small proportion of the digitoxin is bound to pharmacological receptors, the remainder accumulating on non-specific receptors, whereas ouabain is largely bound to specific pharmacological receptors. On the basis of the results of Kuschinsky *et al.* (1968) and of Young (1968), it is probable that procaine accumulates on specific receptors. This view is supported by the observation that, after perfusion of procaine in a concentration sufficient to cause ventricular arrest, subsequent washing out causes a rapid decline in procaine levels, accompanied by an equally rapid disappearance of the pharmacological effect.

If it is permissible to extrapolate from the experiments described in this paper to the antiarrhythmic effect of procaine on the heart in man, it would appear that the short action of procaine in man is probably not wholly due to its rapid metabolism to inactive metabolites, but that the loose binding of procaine to receptors in the heart may be a contributory factor.

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