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A DISCUSSION OF THE MODES OF ACTION OF DRUGS WHICH INCREASE THE CONCENTRATION OF 4-HYDROXY-3-METHOXYPHENYLACETIC ACID (HOMOVANILLIC ACID) IN THE STRIATUM OF THE MOUSE

BY

D. F. SHARMAN

From the Pharmacology Unit, Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge

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The administration to rats of probenecid, an inhibitor of the transport of organic acids in the renal tubules, has been shown to cause an increased concentration in the brain of 5-hydroxyindol-3-ylacetic acid, a metabolite of 5-hydroxytryptamine (Neff, Tozer & Brodie, 1964). Sharman (1966) reported a similar response to probenecid in the mouse and observed a concomitant elevation in the striatum of the concentration of 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid), a metabolite of 3,4-dihydroxyphenylethylamine (dopamine). Werdinius (1966) showed that an increase in the concentration of homovanillic acid also occurred in the brain of the rat after treatment with probenecid. From these observations it has been concluded that there is an active transport mechanism for the removal of these acid metabolites from the brain in these two species. This report is concerned with a method which distinguishes between two types of drug action by which an increase of the striatal concentration of homovanillic acid in the mouse can occur, using the response to a standard dose of probenecid.

METHODS

Female albino mice of a single strain were used. Drugs were administered intraperitoneally, dissolved in a 0.9% w/v solution of sodium chloride. Probenecid was dissolved in the minimum volume of 1N-sodium hydroxide and 0.9% w/v sodium chloride solution was then added. If necessary, the pH of the solution was adjusted to 7–8 with 0.1N-hydrochloric acid. Spiroperidol was dissolved in the minimum volume of glacial acetic acid (10 mg spiroperidol will dissolve in 0.05 ml. glacial acetic acid), the solution was diluted with a little water and final dilutions were made with 0.9% w/v sodium chloride solution. The dissection of the striatal tissues, the extraction and fluorimetric estimation of homovanillic acid were carried out as described by Sharman (1966). The striatal tissue from 2 mice was used for each estimation.

RESULTS

Effect of increasing doses of probenecid on the concentration of homovanillic acid in the striatum of the mouse

The concentration of homovanillic acid in the striatum was estimated 1.5 hr after the administration of increasing doses of probenecid. Control animals were injected with

0.9% w/v sodium chloride solution. The dose-response curve obtained is given in Fig. 1. This shows that the maximum response to probenecid is obtained with a dose of 200 mg/kg.

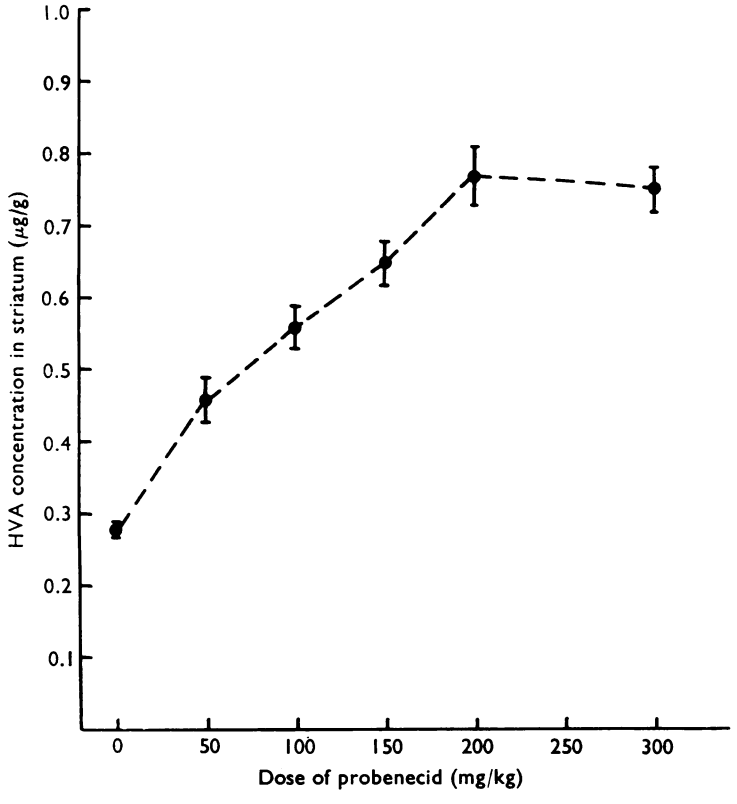


Fig. 1. Dose-response curve for the effect of probenecid on the concentration of homovanillic acid in the striatum of the mouse.

Increase in the concentration of homovanillic acid in the striatum of the mouse produced by a standard dose of probenecid in combination with other drugs

Several types of drugs can cause an elevation of the concentration of homovanillic acid in the striatum of the mouse (Sharman, 1966). The effects on the concentration of striatal homovanillic acid of some of these drugs given alone and in combination with a dose of probenecid (100 mg/kg) are shown in Table 1. The increase due to the administration of probenecid is larger, when this drug is given in combination with 2-aminotetralin (β-tetrahydronaphthylamine), 30 mg/kg; spiroperidol (8-[3-(4-fluorobenzoyl)-propyl]-4-oxo-1-phenyl-1,3,8-triaza-spiro [4,5] decane), 0.5 mg/kg; chlorpromazine, 1 mg/kg and 5 mg/kg, and M99 (6,14-endoetheno-7-(2-hydroxy-2-pentyl)-tetrahydrooripavine hydrochloride), than when given on its own. Spiroperidol (0.1 mg/kg) did not increase the effect of probenecid (100 mg/kg), although this dose of spiroperidol caused a significant increase in the concentration of homovanillic acid when given alone.

TABLE 1

THE EFFECT OF SOME DRUGS GIVEN ALONE AND IN COMBINATION WITH PROBENECID ON THE CONCENTRATION OF HOMOVANILLIC ACID IN THE STRIATUM OF THE MOUSE

Drug (mg/kg)	Concentration of homovanillic acid in the striatum in μg/g tissue ± s.e.m.	With additional probenecid (100 mg/kg)	Difference ± s.d.
Control	0·28±0·01 (9)	0·56±0·03 (18)	0·28±0·04
Probenecid, 100	0·56±0·03 (18)†	0·77±0·04 (9)	0·21±0·05
2-Aminotetralin, 30	0·40±0·03 (9) †	0·91±0·10 (9)	0·51±0·11*
Spiroperidol, 0·1	0·60±0·03 (10)†	0·91±0·05 (10)	0·31±0·07
Spiroperidol, 0·5	0·83±0·02 (10)†	1·42±0·08 (10)	0·59±0·08*
Chlorpromazine, 1	0·40±0·02 (9) †	0·85±0·06 (10)	0·45±0·06*
Chlorpromazine, 5	0·85±0·04 (11) †	1·36±0·04 (11)	0·51±0·05*
M99, 0·2	0·49±0·03 (9) †	0·98±0·07 (9)	0·49±0·07*

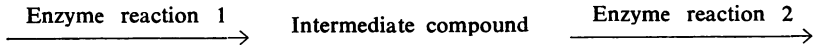
Number of observations for each estimation is given in parentheses.
Drugs in column 1 were given 10–15 min before the probenecid (100 mg/kg).
Estimations were made 1·5 hr after the administration of the probenecid, (100 mg/kg).
* 2 × 2 factorial analysis of variance shows that interaction between drugs is significant $P<0·05$.
† Significantly different $P<0·01$ from control value.

DISCUSSION

The ways in which an increase in the concentration of homovanillic acid in the striatum of the mouse might occur have been discussed by Sharman (1966), but it was not possible to differentiate clearly whether the accumulation was due to an increased rate of formation of the acid or to a reduced rate of its removal from the brain, for only when the rates of removal of other acidic substances were also affected could one feel certain that the mechanism for the removal of homovanillic acid was impaired. The present experiments enable these two mechanisms to be examined.

A simple approach to the processes by which homovanillic acid is formed in and removed from the brain of the mouse is to regard the formation and removal as two consecutive enzyme reactions in an irreversible chain, as shown in Fig. 2, and to assume

Fig. 2.



that probenecid acts by competitive inhibition of the second enzyme. It can be shown (Webb, 1963) that, in such a situation, the concentration of the intermediate compound, in this case homovanillic acid, depends on the rate of its formation and on the ratio of the maximum rates of the two enzyme reactions. Figure 3 shows some theoretically calculated curves for the relationship between the concentration of such an intermediate compound and the rate of the first enzyme reaction and also the effect of changes in the ratio of the maximum rates of the two reactions. From such curves, in the absence of detailed information about conditions prevailing in the mouse striatum, it can be taken as a first approximation that, for low concentrations of the intermediate compound, a two-fold increase in the rate of the first reaction is reflected by a doubling of the concentration of the intermediate.

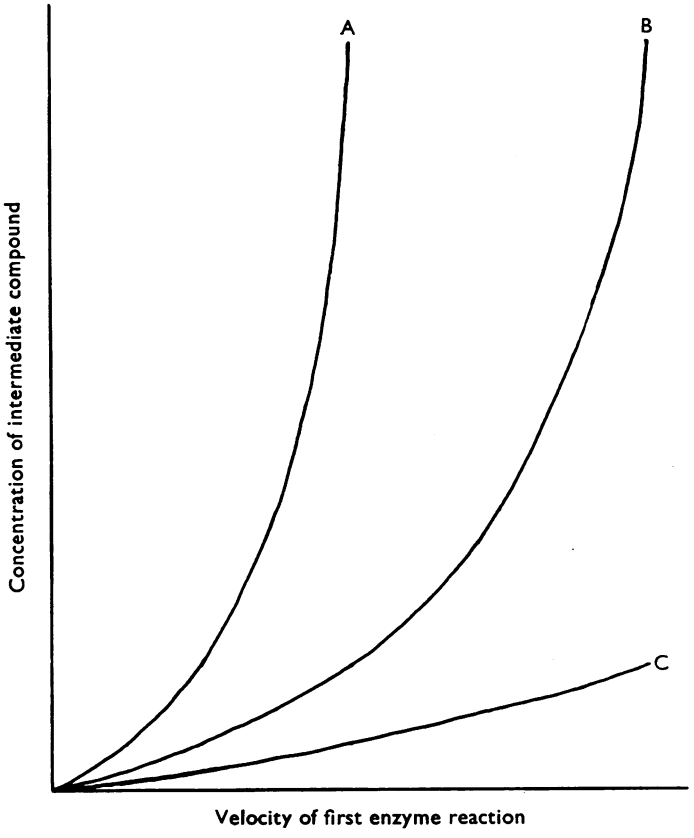


Fig. 3. Typical curves showing the relationship of the concentration of the intermediate compound between two consecutive enzyme reactions to the velocity of the first enzyme reaction.

$$\text{Curve A when } \frac{V_{max} \text{ reaction 1}}{V_{max} \text{ reaction 2}} = 2$$

$$\text{Curve B when } \frac{V_{max} \text{ reaction 1}}{V_{max} \text{ reaction 2}} = 1$$

$$\text{Curve C when } \frac{V_{max} \text{ reaction 1}}{V_{max} \text{ reaction 2}} = 0.5.$$

The increase in the concentration of the intermediate compound when the second enzyme is competitively inhibited can be described (Webb, 1963) by the equation

$$\frac{[B_i]}{[B]} = 1 + \frac{[I]}{K_i} \quad \ldots\ldots (1)$$

where $[B_i]$ = Concentration of intermediate after competitive inhibition of enzyme 2.

$[B]$ = Concentration of intermediate without inhibition of enzyme 2.

[I] = Concentration of competitive inhibitor.

K_i = Dissociation constant for the enzyme-inhibitor complex.

In the present experiments the concentration of the inhibitor, probenecid was kept constant. Under such conditions if the time taken to reach the new level of intermediate does not exceed 1.5 hr, then the ratio $\frac{[B_i]}{[B]}$ should have a constant value and should be independent of the rate of formation of the intermediate compound. If all of the homovanillic acid in the striatum of the mouse is involved in the system which is sensitive to probenecid, then the ratio of the concentration of homovanillic acid in the striatum of mice treated with probenecid to the concentration of this acid in mice not so treated should be equal to $\frac{[B_i]}{[B]}$. If the ratio $\frac{[B_i]}{[B]}$ has the value n then it can be calculated that, in the case where not all of the homovanillic acid in the striatum is involved in the probenecid sensitive transport system, the difference in the striatal concentrations of homovanillic acid between mice treated with probenecid and in similar mice not so treated is equal to n-1 times that portion of the total concentration of homovanillic acid in the mice not treated with probenecid, which will be affected when this inhibitory drug is administered.

i.e.:

From equation (1) when [I] is constant, $\frac{[I]}{K_i} = K$

$$\text{then, } [B_i]-[B] = [B]\,K \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

$$\text{let } \frac{[B_i]}{[B]} = n$$

$$\text{then from equation (1)} \qquad n = 1 + K$$

substituting for K in equation (2) $[B_i]-[B]=(n-1)\,[B]$

let [x] be a part of the initial homovanillic acid concentration unaffected by probenecid, then $[B]+[x]$ increases to $[B_i]+[x]$ after probenecid.

$$([B_i]+[x])-([B]+[x])=[B_i]-[B]=(n-1)\,[B]$$

It is therefore possible to distinguish an increase in the concentration of homovanillic acid due to competitive inhibition of the active transport mechanism, or due to the formation of the acid at a site where this mechanism is not effective, from an increase due to an increased formation of homovanillic acid at the site where it is normally formed, or resulting from non-competitive inhibition of the second enzyme reaction. This latter effect is equivalent to reducing the maximum rate of the second enzyme and the result of such a change can be predicted from the curves given in Fig. 3.

Assuming that all of the homovanillic acid in the striatum of the normal mouse is removed by the probenecid sensitive transport system then, in the present experiments, the value of n calculated from the effect of probenecid in control animals is found to be

2 for a dose of 100 mg/kg. Thus:

1. If a drug increases the concentration of homovanillic acid by competitive inhibition of the active transport system, or by causing the formation of this acid at a site where this system is not effective, then the administration of probenecid (100 mg/kg) in combination with such a drug will produce an additional increase in the concentration of homovanillic acid equal to or smaller than the increase seen after this dose of probenecid given alone.

2. If a drug produces an increase in the concentration of homovanillic acid by an increase in the rate of formation of this acid, or by non-competitive inhibition of the active transport mechanism, then the administration of probenecid (100 mg/kg) in combination with such a drug will produce an additional increase in the concentration of homovanillic acid equal to the concentration of this acid observed in animals treated with the drug alone.

Furthermore, by subtracting the additional increase produced by probenecid (100 mg/kg) from the concentration of homovanillic acid observed in animals treated with the drug alone, it is possible to separate these two types of increase when they occur together.

The increases in the concentration of homovanillic acid seen after a multiple dose of probenecid and after the lower dose of spiroperidol (0.1 mg/kg) correspond with those proposed for the first type of mechanism, the additional increase due to probenecid (100 mg/kg) being approximately equal to the increase seen in the control animals after this dose of probenecid. After the administration of 2-amino-tetralin (30 mg/kg), chlorpromazine (1 mg/kg) and M99 (0.2 mg/kg), the increase has the magnitude required by the second type of mechanism. The additional increase due to probenecid (100 mg/kg) is approximately equal in each case to the concentration observed after treatment with each drug alone. The response to the larger doses of spiroperidol (0.5 mg/kg) and chlorpromazine appears to result from the action of the two types of mechanism. In these two cases the additional increase due to probenecid (100 mg/kg) is smaller than the concentration of homovanillic acid seen in animals treated with these drugs alone. This effect, particularly in the case of chlorpromazine, could be a result of the high rate of formation of homovanillic acid. The time taken to reach the new level of intermediate might be longer than 1.5 hr and thus the increase produced by probenecid would be smaller than expected from the equation.

Using the rate of depletion of dopamine from the mouse striatum after inhibiting the synthesis of this amine in the brain, Dickinson (personal communication) and Sharman (1966) have found evidence that chlorpromazine and spiroperidol cause an increased rate of utilization of dopamine, the amine from which homovanillic acid is formed, but in the case of spiroperidol, the increase in utilization was thought to be insufficient to account for the increase in homovanillic acid produced by this tranquillizing drug. The present results suggest that the action of low doses of spiroperidol in increasing the striatal concentration of homovanillic acid does not involve an increase in the rate of utilization of dopamine through its normal pathway.

An increase in the utilization of dopamine was not observed after treatment with M99 (Sharman, 1966). The possibility that this drug increases the homovanillic acid concentration by non-competitive inhibition of the active transport mechanism must therefore

be considered. The present results yield some indirect evidence that this is not the explanation for the effect of this drug on the concentration of homovanillic acid in the striatum. The dose-response curve relating the striatal concentration of homovanillic acid to the dose of probenecid shows that the concentration of homovanillic acid does not rise above 0.8 μg/g. This could represent the maximum concentration that it is possible to reach in 1.5 hr after total inhibition of the removal of the acid from the brain when the rate of formation of homovanillic acid is normal, and corresponds to a normal turnover rate of the acid of 0.05 μg/g/min.

Treatment with M99 together with probenecid elevated the homovanillic acid to 0.98 μg/g in 1.5 hr, so that it would appear that at least part of the action of M99 is due to an increase in the rate of formation of homovanillic acid in the striatum.

SUMMARY

1. The administration of probenecid, a competitive inhibitor of renal acid transport, to mice results in an increase in the concentration of 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid) in the striatum. This indicates that there is an active acid transport system in this tissue.

2. The effect on striatal homovanillic acid of giving a standard dose of probenecid alone and in combination with other drugs which change the concentration of this acid in the striatum was examined.

3. If the probenecid-sensitive acid transport system in the striatum is regarded as a simple monolinear enzyme chain, then it is possible to distinguish those drugs which increase the concentration of homovanillic acid by competitive inhibition of the acid transport system from those drugs which increase the rate of formation of the acid.

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