J. Pharm. Pharmac, 1969, **21**, 833–837 *Received May* 2, 1969

The influence of the monoamine oxidase inhibitor pargyline hydrochloride on the retention of dopamine in the isolated perfused spleen of the cat

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Much of the dopamine accumulated in the cat spleen after the intravenous infusion of this amine into the anaesthetized animal appears in the venous effluent when the spleen is subsequently isolated and perfused with a dopamine-free medium. Pre-treatment of the animals with the monoamine oxidase inhibitor pargyline hydrochloride has no effect on the rate of decline of the spleen content of dopamine resulting from perfusion, but appears to significantly reduce the amount of dopamine that can be removed from the spleen in this way. The significance of these findings is discussed in relation to a "false" neurochemical transmitter hypothesis for the antihypertensive effects of pargyline.

We have recently shown that perfusion, with a catecholamine-free medium, of cat isolated spleens containing relatively large amounts of endogenous dopamine caused the appearance of dopamine in the venous effluent (Street & Roberts, 1969). The amount of endogenous dopamine washed out declined exponentially with time, a feature which resembled the situation described by Iversen (1965) for the disappearance of adrenaline and noradrenaline from the rat heart following their accumulation by the $Uptake_2$ process. We considered our finding to be evidence for the occurrence of endogenous dopamine in $Uptake_2$ storage sites, which are presumably not "nerve releasable".

We are currently investigating the influence of monoamine oxidase inhibition on the accumulation of dopamine as a "false" neurochemical transmitter. During the course of some of these experiments it was found that when spleens from cats infused with dopamine were isolated and perfused with McEwen solution large amounts of dopamine appeared in the venous outflow.

We have been interested, therefore, in investigating the influence of monoamine oxidase inhibition on the disappearance of accumulated dopamine from the cat isolated perfused spleen.

EXPERIMENTAL

Methods

Male or female cats (2·4 to 3·5 kg) were anaesthetized with chloralose (8 ml/kg of a 1% w/v solution in 0·9% w/v saline) injected into a femoral venous cannula following induction with ether. Some of these cats were pre-treated with pargyline hydrochloride (50 mg/kg) injected subcutaneously 16 h before the experiment.

Dopamine (10 mg/kg) was infused intravenously at a constant rate over 45 min and when the sympathomimetic response had subsided (15 min after terminating the infusion in untreated cats and 90 min after terminating the infusion in pargyline-treated cats), portions of spleen (Dearnaley & Geffen, 1966) were isolated and perfused with McEwen solution (Thoenen, Hurlimann & Haefely, 1963). From some spleen portions venous effluent was collected at various times (up to 70 min) after the start of the perfusion, as 1 min samples in ice-cold centrifuge tubes containing 0·5 ml 2 N HCl, 0·2 ml 5% EDTA solution and a few mg ($\approx$ 2–5) of ascorbic acid. Any red blood cells were removed by centrifugation and proteins were precipitated with perchloric acid at a final concentration of 0·4 N. Dopamine was isolated by modification of the method of Bertler, Carlsson & Rosengren (1958) using Dowex 50 WX-8 cation-exchange resin, 200–400 mesh, hydrogen form, dimensions 7 mm^2 $\times$ 25 mm, and was assayed by the method of Laverty & Sharman (1965). Other spleen portions were homogenized, before perfusion or after 30 min or 60 min perfusion, in 8–16 volumes (depending on the size of the spleen and the volume required) 0·5 N perchloric acid containing a few mg ascorbic acid for 1 min in an Ultra-Turrax homogenizer. EDTA (1 mg/ml) was added to the extracts and dopamine was isolated and assayed as described above. In some experiments phenoxybenzamine hydrochloride (3·4 μg/ml) was added to the perfusing solution.

RESULTS

Dopamine in the venous outflow

The rate at which dopamine (ng/min) was washed into the venous effluent from each spleen portion declined rapidly during the perfusion period (Fig. 1); when this rate was plotted on a log scale a straight line was obtained indicating an exponential decline with time. Experiments with different spleen portions yielded lines of identical slope; pre-treatment of the animals with pargyline or the addition of phenoxybenzamine to the perfusion fluid, or both, failed to modify this slope (Fig. 2).

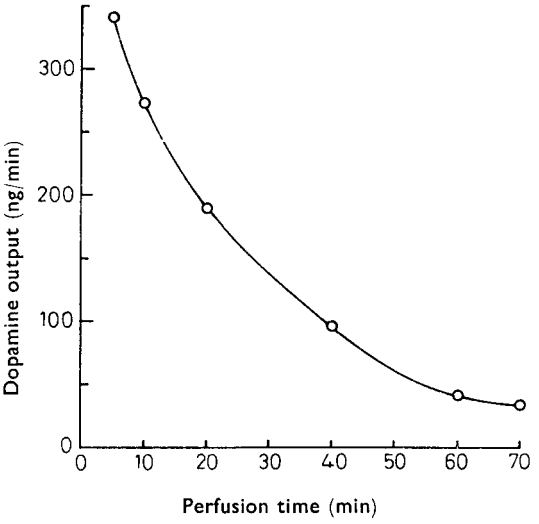

FIG. 1. The relation between the rate of output of dopamine (ng/min) and the time of perfusion of a cat isolated spleen containing dopamine accumulated during an *in vivo* infusion (10 mg/kg over 45 min).

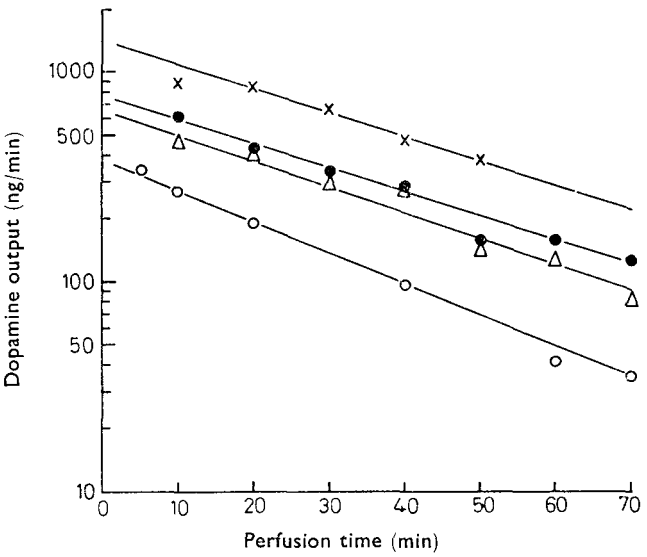

FIG. 2. The relation between the log rate of output of dopamine (ng/min) and the time of perfusion of cat isolated spleens containing dopamine accumulated during an *in vivo* infusion (10 mg/kg over 45 min). Open circles cat pre-treated with, and crosses, cat not pre-treated with, pargyline hydrochloride (50 mg/kg s.c. 16 h before experiment); triangles, cat not pre-treated with pargyline but phenoxybenzamine hydrochloride (3·4 μg/ml) added to the perfusing fluid; closed circles, cat pre-treated with pargyline and phenoxybenzamine added to the perfusing fluid.

Tissue content of dopamine

Perfusion of both untreated and pargyline-treated spleens reduced their dopamine (ng/μmol DNA-P) content (Table 1). Although these spleens contained similar concentrations of dopamine at the start of the perfusion, the amount remaining after 30 min perfusion of pargyline pre-treated spleens was considerably greater than that after similar perfusion of untreated spleens. Experiments after 60 min perfusion confirmed that dopamine was being removed less rapidly from the spleens in the presence of pargyline. In each case, the tissue concentration declined in an exponential

Table 1. *The influence of pargyline hydrochloride on the effect of perfusion with McEwen solution on the dopamine content of cat isolated spleen preparations containing dopamine accumulated during* in vivo *infusions.* Dopamine content in ng/μmol DNA-P. Pargyline hydrochloride (50 mg/kg) administered subcutaneously 16 h before the experiment and dopamine (10 mg/kg) infused intravenously over 45 min. Spleens removed 15 min (untreated cats) and 90 min (pargyline treated cats) after the termination of the dopamine infusions.

Pretreatment	Perfusion time (min)		
	0	30	60
	124·24	44·08	23·56
Saline	146·34	69·89	
	158·95	65·13	
	Mean 143·18	Mean 59·70	
	134·63	71·13	63·69
Pargyline hydrochloride	139·29	91·51	
	141·00	127·73	
	Mean 138·31	Mean 96·79	

manner since plots of the tissue content on a log scale against the perfusion time again resulted in the production of straight lines (Fig. 3). With untreated spleens the slope of this line was identical to that relating dopamine wash-out to perfusion time (Fig. 2), while with pargyline-treated spleens this slope was greatly reduced.

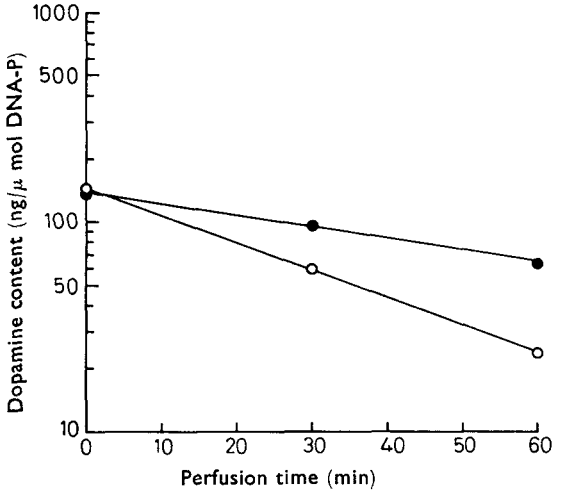

FIG. 3. The relation between the log dopamine content (ng/μmol DNA-P) and the time of perfusion of the cat isolated spleen containing dopamine accumulated during an *in vivo* infusion (10 mg/kg over 45 min). Closed circles, cat pre-treated with, and open circles, cats not pre-treated with, pargyline hydrochloride (50 mg/kg s.c. 16 h before the experiment). All points represent the means of 3 experiments except those after 60 min perfusion which are single observations (data from Table 1).

DISCUSSION

The exponential decline in the rate at which dopamine was washed out in these experiments is similar to that observed for noradrenaline and adrenaline by Iversen (1965) following the accumulation of high perfusion concentrations of both amines by the $Uptake_2$ process in rat isolated heart preparations. It seems not unreasonable, therefore, to assume that the large amount of infused dopamine in the present experiments is being accumulated into similar $Uptake_2$ storage sites in the cat spleen. We have already suggested, for similar reasons, that endogenous dopamine can occur in such sites (Street & Roberts, 1969).

Iversen (1965) observed many similarities between the kinetic and drug susceptibility properties of the $Uptake_2$ process and those of the mechanism by which accumulated amine disappeared during subsequent perfusion with a catecholamine-free medium. He found that phenoxybenzamine inhibited $Uptake_2$ in the rat heart and the experiments of Gillespie & Hamilton (1966) may be taken to indicate that a similar inhibition occurs in the cat spleen. In the present experiments, however, phenoxybenzamine appears not to have an inhibitory effect on the disappearance of accumulated dopamine since the rate constant for the wash-out process was unchanged; by the same token, there is presumably no re-uptake of amine during this wash-out process even in the absence of phenoxybenzamine.

We have found that the application of mathematical equations to the wash-out process simplifies our interpretation of the influence of pargyline in these experiments.

The decline in tissue content of dopamine is thus described by a simple exponential equation of the form:

$$x = ce^{-at} \text{ from which } \log x = \log c - at$$

where x is the tissue content at time t, c is the tissue content at the beginning of the perfusion (when t = 0) and *a* is the rate constant of the wash-out process.

The decline in the rate of wash-out of dopamine into the perfusing fluid is similarly described by an equation of the form

$$\frac{dy}{dt} = ace^{-at} \text{ from which } \log\frac{dy}{dt} = \log ac - at$$

where y is the total amount of dopamine washed into the perfusing fluid after time t.

Since log dy/dt plotted against t (Fig. 2) produces the same rate constant for untreated and pargyline-treated spleens it may be concluded that the wash-out process is not influenced by pargyline. The slopes of the lines from log x against t plots (Fig. 3) however were influenced by pargyline. The slope of this line from experiments with spleens from untreated cats was the same (i.e. equal to *a*) as that of the log dy/dt against t lines, and the disappearance of dopamine from such spleens is therefore in accord with the simple equations given above. With spleens from pargyline-treated cats, however, the slope of the log x against t line was much less than *a* so that the simple equations cannot apply. We feel that the most logical explanation of these findings is that although pargyline has no effect on the mechanism of release from $Uptake_2$ storage sites, the amount of dopamine which can be washed out of the tissue by this process is greatly reduced. Thus if p is assumed to represent a certain amount of dopamine which is not available to the wash-out mechanism, then a linear relation with slope *a* would only be observed between log (x-p) and t in that

$$\log (x - p) = \log (c - p) - at$$

Under these conditions

$$\log x = \log [(c - p)e^{-at} + p] \text{ and } \log\frac{dy}{dt} = \log a(c - p) - at$$

We would postulate, therefore, that the monoamine oxidase inhibitor pargyline hydrochloride changes the location of some of the dopamine accumulated in the cat spleen during an *in vivo* infusion so that it becomes resistant to wash-out. In support of the hypothesis that the antihypertensive effects of monoamine oxidase inhibitors might be associated with the accumulation of dopamine as a weakly sympathomimetic "false" neurochemical transmitter, it is tempting to suggest that this change might represent a subcellular re-distribution of dopamine from non-nerve releasable stores to nerve releasable stores.

Acknowledgement

We are grateful to Abbot Laboratories Ltd. and Smith, Kline & French Laboratories Ltd. for gifts of pargyline hydrochloride and phenoxybenzamine hydrochloride respectively.

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