On the dopaminergic nature of the gnawing compulsion induced by apomorphine in mice

The gnawing compulsion elicited by apomorphine is a consequence of the stimulation of the dopaminergic neurons in the corpus striatum (Ernst & Smelik, 1966; Ungerstedt, Butcher & others, 1969) but the inhibition of the synthesis of dopamine did not alter the response to the drug in rats (Ernst, 1967). Andén, Rubenson & others (1967) demonstrated a diminished turnover rate of dopamine in apomorphine treated rats. The authors concluded that apomorphine acted directly on dopaminergic receptors. Our results show the possibility of an indirect effect of apomorphine in eliciting the gnawing compulsion.

The gnawing was measured in white mice of our Institute's breeding, of either sex, 18–22 g (Ther & Schramm, 1962) in groups of 6, in a cage with a corrugated paper covering the floor. The holes in the paper caused by gnawing, were counted for 10% of the total surface, and the means and s.e. of at least 6 groups of animals were measured.

Reserpine, 0·3–3 mg/kg inhibited the gnawing compulsion caused by 5 mg/kg of apomorphine. The inhibition was dose dependent and statistically significant above the dose of 1 mg/kg ($P < 0{\cdot}02$, Table 1).

Monoamine oxidase inhibitors increased the number of holes caused by gnawing (Table 2). This effect was greatest for AB–15 [1-*m*-aminophenyl-(2-cyclopropylamino)ethanol 2HCl; Huszti, Fekete & Hajós, 1969]. The effect of tranylcypromine was less at 2 mg/kg, but augmenting the dose to 4 mg/kg produced an inhibition of the gnawing compulsion. Nialamide was almost ineffective at 8 mg/kg, while the effect of the dose of 16 mg/kg was equal to that of 4 mg/kg of AB–15, which also caused a reversal of the reserpine induced inhibition (Table 3). The interaction of the monoamine oxidase blocking agent and reserpine elicited an increased sensitivity to apomorphine.

Similar experiments were made in animals treated with dopa and 5-hydroxytryptophan (5-HTP). Neither AB–15, dopa, 5-HTP, nor reserpine caused gnawing

Table 1. *The effect of reserpine on the apomorphine-induced gnawing compulsion of mice*

Treatment 2 h before apomorphine	Treatment at 0 time	No. of groups	Number of gnawed holes ± s.e.
—	Apomorphine 5 mg/kg s.c.	23	193 ± 25
Reserpine 0·3 mg/kg i.p.	Apomorphine 5 mg/kg s.c.	12	162 ± 27
Reserpine 1·0 mg/kg i.p.	Apomorphine 5 mg/kg s.c.	12	78 ± 29
Reserpine 3·0 mg/kg i.p.	Apomorphine 5 mg/kg s.c.	12	66 ± 13

Table 2. *The apomorphine-induced gnawing behaviour as affected by monoamine oxidase inhibitors*

Treatment	Pretreatment time (h)	Number of gnawed holes after apomorphine (number of groups used) at: 2·5 mg/kg	5·0 mg/kg
Tranylcypromine 2 mg/kg i.p.	4	155 ± 23 (12)	297 ± 41 (11)
AB-15 4 mg/kg i.p.	18	174 ± 28 (17)	350 ± 31 (18)
Nialamide 16 mg/kg i.p.	18	256 ± 16 (10)	322 ± 7(10)
Dist. water 20 ml/kg i.p.	—	139 ± 15 (16)	193 ± 25(23)

Table 3. *The effect of dopa, 5-HTP, reserpine and AB–15 on the apomorphine-induced gnawing behaviour*

Pretreatment	Treatment	Number of groups	Number of holes gnawed
AB-15 4 mg/kg —18 h	Apomorphine 5 mg/kg s.c.	18	350 ± 31
Reserpine 1 mg/kg — 1 h	Apomorphine 5 mg/kg s.c.	12	147 ± 32
AB-15 4 mg/kg + Reserpine 1 mg/kg	Apomorphine 5 mg/kg s.c.	6	325 ± 75
Dopa 25 mg/kg i.p. — 1 h	Apomorphine 5 mg/kg s.c.	12	266 ± 70
Dopa 25 mg/kg	Apomorphine 5 mg/kg s.c.	7	408 ± 53
+ AB-15 4 mg/kg		23	193 ± 25
—	Apomorphine 2.5 mg/kg s.c.	16	139 ± 15
AB-15 4 mg/kg	Apomorphine 2.5 mg/kg s.c.	17	174 ± 28
5-HTP 12 mg/kg — 1 h	Apomorphine 2.5 mg/kg s.c.	8	97 ± 27
AB-15 4 mg/kg + 5-HTP 12 mg/kg	Apomorphine 2.5 mg/kg s.c.	7	258 ± 35

compulsion in the doses used. The precursor amino-acids alone did not change significantly the gnawing behaviour caused by apomorphine. In animals given AB–15, dopa elicited a marked hypersensitivity to apomorphine. Similar effect was seen in AB–15 treated animals when 5-HTP was injected before apomorphine administration, the dose of 2·5 mg/kg of apomorphine caused an effect greater than that of 5 mg/kg in control animals (Table 3).

These results show that the apomorphine-induced stimulation of the corpus striatum may be indirect in nature. Making more dopamine or 5-hydroxytryptamine available equally leads to a greater apomorphine effect; in contrast, the depletion of these amines caused by reserpine leads to an inhibition of the gnawing. Ther & Schramm (1962) and Andén & others (1967) could not show the inhibition of apomorphine-gnawing by reserpine, possibly because of the adverse pharmacologic effects of the high doses used. Similarly Ther & Schramm (1962) demonstrated an inhibition of the apomorphine-induced gnawing by a high dose of iproniazid, while in our experiments the enzyme inhibitors we used increased it.

It is not easy to relate our answers to those which show that apomorphine retards the utilization of dopamine in the brain (Andén & others, 1967; Roos, 1969; Butcher & Andén, 1969). Bearing in mind the two compartmental system of the storage and metabolism of catecholamines suggested by Sedvall, Weise & Kopin (1968), there are many ways of changing the metabolism of transmitter amines, in addition to the positive or negative feed back regulation of the synthesis. A delayed depletion after α-methyltyrosine may not be the obligatory consequence of a diminished utilization.

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February 10, 1970

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Pharmacological actions of pralidoxime in relation to therapeutic doses

Several years ago we referred (Berry, Davies & Rutland, 1966) to speculations that pyridinium aldoximes, used as antidotes to organophosphorus anticholinesterase poisoning, might exert a biphasic action at neuromuscular junctions, but pointed out that none of these speculations were accompanied by measurements of the concentration of oxime reached in end-plates after the administration of therapeutic amounts of the drugs. Berry & others (1966) found that the maximum concentration of TMB-4 [1,3-di(4-hydroxyiminomethylpyridinium)propane dichloride] in diaphragm muscle did not exceed 0·1 mmol/kg with therapeutic doses, but higher concentrations were produced by toxic doses. Goyer (1970) has recently revived these speculations about a biphasic action by showing that concentrations of PAM (pyridine-2-aldoxime methiodide) around 1 mM caused optimal stimulation of the release of acetylcholine from the rat phrenic-diaphragm preparation. Concentrations of drug reached *in vivo* are too low to stimulate the release of acetylcholine, and hence oxime-induced release of this substance could have no influence on the therapeutic action of the drug (Table 1).

Table 1. *Concentration of P2S in the diaphragm after intramuscular injection of 30 mg/kg.* Values, in μg/g fresh weight, are the mean of six observations (i.e. 36 animals of each species)

	Time after injection, min					
	5	10	20	40	60	90
Guinea-pig ..	10·2	15·0	17·7	13·2	11·0	8·6
Rat	12·2	16·8	20·3	21·0	25·7	12·2

A dose of 30 mg/kg of P2S (pyridine-2-aldoxime methylmethanesulphonate) affords excellent protection against organophosphates when used in conjunction with atropine (Davies & Willey, 1959). Rats or guinea-pigs were given this dose intramuscularly, and groups of six animals were killed at intervals thereafter. The concentration of P2S in the diaphragm was measured by a modification of the method of Creasey & Green (1959). The Table shows that the peak concentration reached in rat diaphragm was 26 μg/g, or about 0·11 mmol/kg calculated as pyridine-aldoxime methylmethane sulphonate. According to Goyer's (1970) data this concentration would not significantly alter the release of acetylcholine from the muscle.

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