Br. J. Pharmac. (1975), **54**, 157-162

ROLE OF CATECHOLAMINES IN THE CENTRAL MECHANISM OF EMETIC RESPONSE INDUCED BY PERUVOSIDE AND OUABAIN IN CATS

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1 Peruvoside, (a glycoside obtained from the plant, *Thevetia neriifolia Juss*) and ouabain produce emesis in cats. Vomiting is not produced by these drugs in animals pretreated with catecholamine depleting drugs like reserpine, tetrabenazine or syrosingopine. Chloropromazine hydrochloride, mepyramine maleate, or BOL-148 administered intravenously or intracerebroventricularly do not afford protection.

2 Phenoxybenzamine produces partial protection against peruvoside-induced emesis.

3 Haloperidol (1 mg/kg i.v.) prevents vomiting induced by peruvoside or ouabain. Intracerebroventricularly administered haloperidol is ineffective.

4 Cats pretreated with SKF-525-A, are not protected by haloperidol. Animals pretreated with phenobarbitone in a dose of 25 mg/kg for a week were protected by haloperidol, 250 μg/kg i.e. one quarter of the effective antiemetic dose in normal cats.

5 It is postulated that catecholamines are involved in the mechanism of vomiting induced by cardiac gycosides. Further, a metabolite of haloperidol seems to be responsible for its effective antiemetic action.

Introduction

The chemoreceptor trigger zone in the area postrema has been shown to be the central site of emetic response induced by a number of drugs (Borison & Brizzee, 1951; Wang & Borison, 1952). This area is rich in catecholamines (Vogt, 1954). Borison (1959) and Feldberg & Sherwood (1954) have shown that intracerebroventricular administration of catecholamines induced emesis in dogs and cats. Cahen (1964) reported that 5-hydroxytrytophan-induced emesis in cats was brought about through the release of catecholamines. Koppanyi & Cowan (1962) reported catecholamines as possible stimulants of chemoreceptor mechanisms. Peng (1963) has shown that dihydroxyphenylalanine (DOPA) induced emesis in dogs. We have investigated the role of catecholamines in the mechanism of the emetic response induced by ouabain and peruvoside. In our investigation, we found that haloperidol possessed a potent antiemetic activity and it was decided to investigate further the mechanism of this action of haloperidol.

Methods

Cats of either sex weighing between 2-4 kg were used. Various doses of peruvoside or ouabain were administered via the saphanous vein by a rapid injection in volumes not exceeding 3 ml.

A cannula was placed in the lateral cerebral ventricle according to a method described by Feldberg &.Sherwood (1954). The position of the cannula was verified at the end of each experiment. All emetic tests were performed shortly after feeding. Expulsion of gastric contents was taken as a positive response. The functional integrity of the vomiting mechanism was confirmed after each experiment by oral administration of copper sulphate (20 mg in 50 ml of water) (Wang & Borison, 1952).

In order to explore the role of catecholamines, various catecholamine depleting drugs or antagonists were used. The doses, route and the time of challenge are given in Table 1.

To assess the role of microsomal enzymes, a group of 20 cats, received SKF-525-A (βdiethylaminoethyl diphenylpropyl acetate hydrochloride) in a dose of 75 mg/kg intraperitoneally and the animals were challenged with peruvoside or ouabain after 6 and 24 hours.

Sodium phenobarbitone in a dose of 25 mg/kg was administered intraperitoneally daily, for eight days in 18 cats and the animals were challenged with peruvoside or ouabain.

Drugs

SKF-525-A, was supplied by Smith Kline and French Laboratory, Philadelphia; reserpine and syrosingopine were supplied by Ciba Research Center, India. Tetrabenazine, was supplied by Roche Pharmaceuticals India, haloperidol and pimozide were gifts from Janssen Pharmaceuticals, Belgium. Peruvoside was supplied by the Director, Indian Council of Medical Research, New Delhi, India. Other drugs were obtained from local commercial firms.

Results

Emetic response in cats

The results of administration of peruvoside and ouabain are presented in Table 2. In cats the maximum effective emetic dose of peruvoside was 125 μg/kg given intravenously. The latency of vomiting by the intravenous route was 3-8 min (average 5.5 minutes). A dose of 125 μg/kg intravenously was lethal in 4 out of 20 cats, but the vomiting was not the terminal event. Since this was the most effective dose, it was used in all subsequent experiments. The most effective emetic dose of ouabain was found to be 60 μg/kg intravenously and this was used for comparison.

All cats which vomited had anorexia, lasting for 2-5 days.

Emetic response in cats treated with reserpine, tetrabenazine and syrosingopine

Table 3 illustrates the effect of these catecholamine-depleting drugs, given in the doses shown in Table 1, on emetic response induced by peruvoside or ouabain. It can be seen that chronic reserpine-treatment or intracerebroventricular reserpine abolished peruvoside as well as ouabain-induced emesis. The cats were sedated. Cats treated with tetrabenazine (50 mg/kg i.v.) or syrosingopine (10 mg/kg i.v.) did not vomit after administration of peruvoside or ouabain. None of the animals in these groups showed marked sedation. All animals who failed to vomit after peruvoside or ouabain vomited after administration of copper sulphate orally, with a latency of 12-20 min (average 16 minutes).

Table 1 Schedule of treatment of cats challenged with peruvoside or ouabain.

Treatment	*Dose and Route*	*No. of cats*	*Time of challenge*
Reserpine	*1st day* 2 mg/kg i.m. *2nd day* 0.5 mg/kg i.m. *3rd day* 1 h prior to challenge, 0.3 mg/kg i.m.	12	3rd day
Reserpine	500 μg i.c.v. in divided doses	6	4 h
Syrosingopine	10 mg/kg i.v.	12	24 h
Tetrabenazine	50 mg/kg i.v.	12	4 h
Phenoxybenzamine	5 mg/kg i.v.	15	0.5 h
	2 mg i.c.v. in divided doses	11	1 h
Chlorpromazine	2.5 mg/kg i.v.	12	1 h
	750 μg i.c.v. in divided doses	6	0.5 h
Mepyramine maleate	10 mg/kg i.m.	12	1 h
	3 mg i.c.v. in divided doses	6	40 min
BOL—148	50 μg/kg i.v.	12	0.5 h
	20 μg i.c.v.	6	0.5 h
Haloperidol	1 mg/kg i.v.	20	1 h
	250 μg/kg i.v.	28	1 h
	200 μg i.c.v. in divided doses	6	0.5 h
Pimozide	1 mg/kg i.v.	3	24 h

i.v. = intravenous; i.c.v. = intracerebroventricular; i.m. = intramuscular.

Effect of mepyramine maleate, BOL-148, chlorpromazine hydrochloride and phenoxybenzamine

Mepyramine maleate, BOL-148, chlorpromazine hydrochloride intravenously or intracerebroventricularly prior to emetic challenge did not prevent emesis (Table 4).

Phenoxybenzamine afforded protection in 50% of animals challenged with peruvoside.

Antiemetic action of haloperidol and pimozide

Haloperidol given in a dose of 1 mg/kg intravenously, 1 h before the emetic challenge antagonized the action of peruvoside and ouabain. All such cats rendered refractory to emetic action of peruvoside or ouabain by haloperidol, vomited after oral administration of copper sulphate. The results are presented in Table 5.

Haloperidol, 200 μg introduced into the lateral ventricle 30 min before peruvoside or ouabain were administered intravenously, failed to protect the animals against vomiting induced by these drugs, although the drug produced a moderate degree of sedation. Intracerebroventricular haloperidol itself produced vomiting in all the cats

Table 2 Emetic response after intravenous administration of peruvoside and ouabain in cats.

Drug	*Route*	*Dose (μg/kg)*	*No. of cats vomited/tested*	*Latency in min (range)*
Peruvoside	i.v.	50	0/5	–
		75	2/5	20-69
		100	3/5	20-40
		125	20/20*	3-8
		150	5/5**	3-6
Ouabain	i.v.	60	15/15	8-22

i.v. = intravenous; * = four animals died; ** = all animals died.

Table 3 Effect of reserpine, tetrabenazine and syrosingopine on emesis in cats induced by peruvoside and ouabain.

Drug	*Dose (μg/kg i.v.)*	*Control*	*No. of cats vomited/No. of cats tested* *Reserpine* *i.m.*	*i.c.v.*	*Tetrabenazine* *i.v.*	*Syrosingopine* *i.v.*
Peruvoside	125	20/20	0/6	0/3	0/6	0/6
Ouabain	60	15/15	0/6	0/3	0/6	0/6
Copper sulphate 20 mg in 50 ml of water	orally	8/8	12/12	6/6	12/12	12/12

i.v. = intravenous; i.c.v. = intracerebroventricular.
Doses of reserpine, tetrabenazine and syrosingopine given in Table 1.

Table 4 Effect of mepyramine maleate, BOL–148, phenoxybenzamine and chlorpromazine on emesis in cats induced by peruvoside and ouabain.

Drug	*Dose μg/kg*	*Control*	*No. of cats vomited/No. of cats tested* *Mepyramine maleate* *i.m. 10 mg/kg*	*i.c.v. 3 mg*	*BOL–148* *i.v. 50 μg/kg*	*i.c.v. 20 μg*	*Phenoxybenzamine* *i.v. 5 mg/kg*	*i.c.v. 2 mg*	*Chlorpromazine* *i.v. 2.5 mg/kg*	*i.c.v. 750 μg*
Peruvoside	125	20/20	6/6	3/3	6/6	3/3	5/10	5/5	6/6	3/3
Ouabain	60	15/15	6/6	3/3	6/6	3/3	5/5	6/6	6/6	3/3
Copper sulphate 20 mg in 50 ml of water	orally	8/8	–	–	–	–	5/5	–	–	–

i.v. = intravenous; i.c.v. = intracerebroventricular; i.m. = intramuscular.

with an average latency of 3 min (range 2-4 minutes).

Pimozide in a dose of 1 mg/kg intravenously, 24 h prior to emetic challenge protected the cats against peruvoside-induced emesis. All cats rendered refractory to the emetic action, vomited after oral administration of copper sulphate.

Effect of prior treatment with SKF-525-A The effects of haloperidol (1 mg/kg i.v.) given 6 h and 24 h after administration of SKF-525-A (75 mg/kg i.p.) are presented in Table 6. The most effective antiemetic dose of haloperidol (1 mg/kg i.v.) failed to protect about 30% of the animals at 6 hours. The antiemetic action of haloperidol was not seen in SKF-525-A treated animals when haloperidol was administered 24 h after SKF-525-A treatment; all animals in the group which received ouabain and 6 out of 7 cats in the group challenged with peruvoside vomited although they had received haloperidol just prior to emetic challenge.

Effect of phenobarbitone treatment The effect of chronic administration of sodium phenobarbitone on the antiemetic action of haloperidol is shown in Table 7.

Haloperidol in a dose of 250 μg/kg intravenously did not have any antiemetic action in normal animals. However, after pretreatment with phenobarbitone this dose of haloperidol afforded protection in 50% of the animals when challenged with the emetic dose of either peruvoside or ouabain.

Discussion

The role of catecholamines in the vomiting mechanism is not clearly defined. High concen-

Table 5 Antiemetic action of haloperidol in cats.

Drug	*Dose (μg/kg i.v.)*	*Control*	*No. of cats vomited/No. of cats tested* *Haloperidol-treated* *i.v. (1 mg/kg)*	*i.c.v. (200 μg)*	*Pimozide-treated* *i.v. (1 mg/kg)*
Peruvoside	125	20/20	0/10	3/3	0/3
Ouabain	60	15/15	0/10	3/3	–
Copper sulphate 20 mg in 50 ml of water	orally	8/8	20/20	–	3/3

i.v. = intravenous; i.c.v. = intracerebroventricular.

Table 6 Effect of SKF-525-A treatment on the protective action of intravenous haloperidol against vomiting in cats induced by peruvoside and ouabain.

Drug	*Dose (μg/kg)*	*Route*	*No. of cats vomited/No. of cats tested** *6 h*	*24 h*
Peruvoside	125	i.v.	1/3	6/7
Ouabain	60	i.v.	1/3	7/7

* = All animals received SKF-525-A, (75 mg/kg i.p.) followed by haloperidol (1 mg/kg i.v.) (see text).

Table 7 Effect of sodium phenobarbitone treatment on the antiemetic action of intravenous haloperidol in cats.

Drug	*Dose (μg/kg)*	*Route*	*No. of cats vomited/No. of cats tested* *Haloperidol (250 μg/kg i.v.)* *Before treatment*	*After treatment**
Peruvoside	125	i.v.	5/5	5/10
Ouabain	60	i.v.	5/5	4/8

* = Animals treated with sodium phenobarbitone (25 mg/kg i.p.) for 8 days prior to challenge.

tration of amines occurs in the area postrema (Vogt, 1954). Adrenaline and its precursors potentiate apomorphine-induced emesis (Boyd & Cassel, 1957; Forster & Gunther, 1962). Further, catecholamine depleting drugs have been reported to afford protection against emesis induced by 5-hydroxytryptophan (Cahen, 1964), apomorphine (Boyd & Cassel, 1957), and staphylococcal enterotoxin (Sugiyamma, Bergdoll & Wilkerson, 1960).

Our results indicate that reserpine, tetrabenazine and syrosingopine abolish vomiting induced by peruvoside and ouabain. Since reserpine depletes catecholamines from both central and peripheral adrenergic neurones (Carlsson, Rosengren & Nilsson, 1957), the role of catecholamines was further analysed by the use of intravenous tetrabenazine or syrosingopine and intraventricular reserpine. Tetrabenazine has been shown to exert most of its actions centrally (Quinn, Shore & Brodie, 1959). The results obtained by treatment with tetrabenazine intravenously or intracerebroventricular reserpine indicate that the depletion of catecholamines from the brain is responsible for antagonizing the emesis induced by peruvoside and ouabain. The protective action of these drugs does not seem to be due to sedation of the animal, since all cats that failed to vomit after peruvoside or ouabain readily vomited after oral copper sulphate indicating the emetic mechanism or threshold to copper sulphate was unimpaired.

Our results with syrosingopine are intriguing. Syrosingopine has been shown to deplete catecholamines peripherally (Orlans, Finger & Brodie, 1960). It antagonized both peruvoside and ouabain-induced vomiting. In our experiments ouabain has been shown to produce vomiting by activating the chemoreceptor trigger zone (Borison & Brizzee, 1951), while peruvoside induces vomiting by an action on the nodose ganglion (Gaitonde & Joglekar, 1972). The antiemetic action of syrosingopine in peruvoside-induced emesis could well be due to depletion of amines in the nodose ganglion which has been reported to be rich in adrenergic neurones (Koelle, 1955). The effectiveness of syrosingopine in ouabain-induced emesis may be due to depletion of catecholamines in the chemoreceptor trigger zone.

Phenoxybenzamine afforded only partial protection against peruvoside-induced emesis; five out of ten animals treated with the blocking agent did not vomit. Further, the drug was totally ineffective against ouabain-induced emesis. Lack of protection after phenoxybenzamine in this case may indicate that the drug did not reach the receptor site. Alternatively more than one transmitter mechanism may be involved in the integrated process of vomiting. BOL-148 and antihistamines were ineffective in peruvoside- and ouabain-induced vomiting, suggesting that 5-hydroxytryptamine and histamine do not play a role in the mechanism of vomiting. Chlorpromazine, although it produced a fair degree of sedation, failed to abolish the emetic action of either ouabain or peruvoside.

Haloperidol (1 mg/kg i.v.) abolished peruvoside- and ouabain-induced emesis, in spite of the fact that the animals received the maximal effective emetic dose. Although haloperidol produced sedation, impairment of emesis did not seem to be due to this action. The vomiting mechanism in these animals is not impaired, as can be seen from the fact that all the haloperidol-treated animals vomited after oral copper sulphate. Haloperidol is thus an effective antiemetic agent in peruvoside- and ouabain-induced emesis.

One fifth of the effective intravenous antiemetic dose of haloperidol when given intracerebroventricularly, failed to protect the animals from emesis induced by peruvoside and ouabain. This may indicate that intracerebroventricular haloperidol may not reach the receptor site. However, another possibility is that a metabolite of the drug may be responsible for the antiemetic activity of haloperidol. Such a possibility is suggested by our experiments, in which microsomal enzyme inhibitors or inducers were used. Thus in cats pretreated with SKF-525-A, a known microsomal enzyme inhibitor (Brodie, 1956) the protective action of haloperidol was completely lost, when the drug was administered 24 h after SKF-525-A treatment, with some effect possibly being present, even at 6 h after treatment with SKF-525-A. Further evidence for implication of a metabolite of haloperidol is provided by our experiments in which phenobarbitone, a microsomal enzyme inducer (Brodie, 1956) was used. After chronic treatment of cats with sodium phenobarbitone, haloperidol in a dose as small as 250 μg/kg intravenously, was found to produce an antiemetic action in 50% of the cats treated. This dose of haloperidol failed to protect normal animals (Table 7). Thus both the microsomal enzyme inhibitor as well as the inducer influenced the antiemetic action of haloperidol in a predictable manner. We therefore postulate that a metabolite of haloperidol in cats is probably an effective antiemetic agent.

Haloperidol is a weak adrenoceptor blocking drug but a strong dopamine antagonist (Fuxe & Sjoqvist, 1972; Van Rossum, 1966). Apomorphine has also been shown to stimulate dopamine receptors. Haloperidol seems to protect effectively against apomorphine- and digitalis-induced emesis and it is therefore possible that digitalis- as well as

apomorphine-induced emesis, may be due to activation of dopamine receptors. Pimozide has been reported to be a specific dopamine antagonist, (Fuxe & Sjoqvist, 1972). Cats treated with pimozide (1 mg/kg) 24 h prior to emetic challenge, were totally protected. This lends further evidence to the possibility of involvement of dopamine receptors in the nodose ganglion and chemoreceptor trigger zone responsible for emesis induced by peruvoside or ouabain. Since one of the earliest manifestations of digitalis toxicity is vomiting, haloperidol and pimozide may be useful clinically in the treatment of vomiting due to digitalis toxicity.

The authors wish to thank The Director, Central Council for Research in Indian Medicine and Homoeopathy, New Delhi, India, for financial assistance and Dr P. Janssen, Janssen Pharmaceutica, Belgium for the generous supply of haloperidol and pimozide.

References

BORISON, H.L. (1959). Effect of ablation of medullary emetic chemoreceptor trigger zone on vomiting responses to cerebral intraventricular injection of adrenaline, apomorphine and pilocarpine in the cats. *J. Physiol. Lond.*, **147**, 172-177.

BORISON, H.L. & BRIZZEE, K.R. (1951). Morphology of emetic chemoreceptor trigger zone in the cats medulla. *Proc. Soc. exp. Biol. Med.*, 77, 38-42.

BOYD, E.M. & CASSEL, W.A. (1957). Agents affecting apomorphine induced vomiting. *J. Pharmac. exp. Ther.*, **119**, 390-394.

BRODIE, B.B. (1956). Pathways of drug metabolism. *J. Pharm. Pharmac.*, 8, 1-16.

CAHEN, R.L. (1964). On the mechanism of emesis induced by 5-hydroxytryptamine. *Proc. Soc. exp. Biol. Med.*, **116**, 402-404.

CARLSSON, A., ROSENGREN, E. & NILSSON, S. (1957). Effect of reserpine on the metabolism of catecholamines. In *Psychotropic Drugs* pp. 363-372. ed. Garattini, S. & Ghetti, V. Amsterdam: Elsevier.

FELDBERG, W. & SHERWOOD, S.L. (1954). Injection of drugs into the lateral ventricle of the cat. *J. Physiol., Lond.*, **123**, 148-167.

FORSTER, W. & GUNTHER, E. (1962). Die Beeinflussung des apomorphiner Breches durch Pervitin, Iproniazid und Catecholaminvorstufer. *Acta Biol. med. Germ.*, **8**, 464-471.

FUXE, K. & SJOQVIST, F. (1972). Hypothermic effect of apomorphine in the mouse. *J. Pharm. Pharmac.*, **24**, 702-705.

GAITONDE, B.B. & JOGLEKAR, S.N. (1972). Mechanism of peruvoside induced emesis. *Neuropharmacology*, **11**, 427-433.

KOELLE, G.B. (1955). The histological identification of acetylcholinesterase in cholinergic, adrenergic and sensory neurons. *J. Pharmac, exp. Ther.*, **114**, 167.

KOPANYI, T. & COWAN, F.F. (1962). Catecholamines as possible stimulants of chemoreceptor mechanisms. *Arch. int. Pharmacodyn. Thér., CXXXIX*, 3-4, 564-571.

ORLANS, F.B., FINGER, K.F. & BRODIE, B.B. (1960). Pharmacological consequences of the selective release of peripheral stores of norepinepherine by syrosingopine (S.U. 3118). *J. Pharmac. exp. Ther.*, **128**, 231-239.

PENG, M.T. (1963). Locus of emetic action of epinephrine and DOPA in dogs. *J. Pharmac. exp. Ther.*, **189**, 345-349.

QUINN, G.P., SHORE, P.A. & BRODIE, B.B. (1959). Biochemical and pharmacological studies of R.O.1-9569 (tetrabenazine), a non-indole tranquilizing agent with reserpine-like effect. *J. Pharmac. exp. Ther.*, **127**, 103-109.

SUGIYAMMA, H., BERGDOLL, M.S. & WILKERSON, R.G. (1960). Perphenazine and reserpine as an antiemetic for staphylococcal enterotoxin. *Proc. Soc. exp. Biol. Med.*, **103**, 168-172.

VAN ROSSUM, J.M. (1966). The significance of dopamine receptor blockade for the mechanism of action of neuroleptic drugs. *Arch. Int. Pharmacodyn. Thér.*, **160**, 492-493.

VOGT, M. (1954). The concentration of sympathin in the different parts of the central nervous system under normal conditions and after administration of drugs. *J. Physiol., Lond.*, **123**, 451-481.

WANG, S.C. & BORISON, H.L. (1952). A new concept of organisation of the central emetic mechanism. Recent studies on the sites of action of apomorphine, copper sulphate and cardiac glycosides. *Gastroenterology*, **22**, 1, 1-12.