Kadlec, O., Mašek, K., Šeferna, I. (1979) Eur. J. Pharmacol. 60: 383–386

Kadlec, O., Mašek, K. (1979) in: Tuček, S. (ed.) Cholinergic Synapse. Elsevier, Amsterdam, pp 203–209

Kadlec, O., Šeferna, I., Mašek, K., Somogyi, G. T., Knoll, J. (1982) Eur. J. Pharmacol. 79: 245–255

MacIntosh, F. C., Collier, B. (1976) in: Zaimis, E. (ed.) Neuromuscular Junction. Springer-Verlag, Berlin, p. 187

Northover, B. J. (1971) Br. J. Pharmacol. 41: 540–551

Paton, W. D. M., Vizi, E. S., Zar, M. A. (1971) J. Physiol. (London) 215: 819–848

Piper, P. J., Vane, J. R. (1971) Ann. N.Y. Acad. Sci. 180: 363–368

Puig, M.M., Gascón, P., Musacchio, J. M. (1978) J. Pharmacol. Exp. Ther. 206: 289–302

Vane, J. R. (1971) Nature (New Biol.) 231: 232–235

Yagasaki, O., Takai, M., Yanagiya, I. (1981) J. Pharm. Pharmacol. 33: 521–525

J. Pharm. Pharmacol. 1984, 36: 68–70
Communicated June 6, 1983

Evidence that drugs increasing 5-hydroxytryptamine transmission block jumping but not wet dog shakes in morphine-abstinent rats: a comparison with clonidine

S. ROMANDINI, L. CERVO, R. SAMANIN*, *Istituto di Ricerche Farmacologiche 'Mario Negri' Via Eritrea, 62-20157 Milano, Italy*

(+)-Fenfluramine, a 5-hydroxytryptamine (5-HT) releaser and uptake blocker, *m*-chlorophenylpiperazine (CPP), a 5-HT receptor agonist, and clonidine, an agonist at adrenoceptors, were studied for their ability to modify jumping and wet dog shakes in morphine abstinent rats. (+)-Fenfluramine and CPP blocked jumping with no effect on wet dog shakes whereas the reverse was true for clonidine. The results further show that 5-HT mechanisms are preferentially involved in the expression of jumping in morphine-abstinent rats.

Recent studies have shown that drugs which increase 5-hydroxytryptamine (5-HT) transmission inhibit naloxone-precipitated jumping in morphine-dependent rats, with little or no effect on other signs such as ptosis and diarrhoea (Samanin et al 1980; Cervo et al 1981).

Wet dog shakes, commonly studied as a withdrawal sign (Wei et al 1973; Lal & Numan 1975; Vetulani & Bednarczyk 1977), were not measured in these studies since they were present to a very limited extent in morphine abstinent rats. This may have been the result of the high level of morphine dependence used since it has been shown that the frequency of wet dog shakes is inversely related to the intensity of morphine dependence (Bläsig et al 1973).

The present study examined the effects of (+)-fenfluramine, a 5-HT releaser and uptake blocker (Garattini et al 1975) and *m*-chlorophenylpiperazine (CPP), a 5-HT receptor agonist (Samanin et al 1979), on wet dog shakes in two different naloxone-precipitated withdrawal syndromes where this sign is well represented. The effects were compared with that of clonidine, an agonist at adrenoceptors (Andén et al 1970), which has been found to inhibit wet dog shakes in rats (Tseng et al 1975) and some aspects of the morphine abstinence syndrome in man (Gold et al 1978).

* Correspondence.

Materials and methods

Male CD-COBS rats (Charles River, Italy) about 200 g at the beginning of the experiments, were housed (3 per cage) under constant room temperature (21 + 1 °C) and relative humidity (50%) with a 12 h light −12 h dark cycle (dark period commencing at 19.30). Food and water were freely available.

Induction of physical dependence. One group of rats (Exp. A) was made dependent by subcutaneous implantation of a pellet containing 75 mg morphine free base formulated according to the method of Gibson & Tingstad (1970). Withdrawal was tested 72 h after implantation. Another group of animals (Exp. B) received two intraperitoneal injections on day 1 (9 am and 6 pm) of 5 mg kg^{-1} morphine hydrochloride, calculated as free base. The dose of morphine was doubled every day thereafter to reach a total daily dose of 40mg kg^{-1} on day 4. At 9 am on day 5 the animals received the last injection of morphine (25 mg kg^{-1}) and were tested for withdrawal 4 h later.

Testing for withdrawal. The abstinence syndrome was precipitated in groups A and B with an intraperitoneal injection of 1·0 mg kg^{-1} naloxone HCl, dissolved in distilled water. Pellets in group A were not removed before testing. Before injection of naloxone, the animals were placed individually for 30 min acclimatization in test chambers consisting of rectangular Acryglass boxes (30 × 30 × 25 cm). Equal numbers of controls and experimental animals for each treatment were tested simultaneously.

Withdrawal signs within 30 min were recorded by observers uninformed of an animal's treatment conditions. Abstinence signs precipitated by naloxone in both experimental groups consisted mainly of wet dog shakes, diarrhoea, ptosis and, to a lesser extent, jumping. Other signs such as teeth chattering, vocaliza-

Table 1. Effect of (+)-fenfluramine, *m*-chlorophenylpiperazine (CPP) and clonidine on wet dog shakes and jumping in morphine-dependent rats.

Treatment	mg kg^{-1}	Wet dog shakes No. of episodes (mean ± s.e.)	Wet dog shakes Positive rats (%)	Jumping No. of episodes (mean ± s.e.)	Jumping Positive rats (%)
Pellet implantation					
Saline	—	4·5 ± 0·9	68	4·7 ± 1·6	50
(±)-Fenfluramine	5	4·5 ± 1·0	71	0·0‡	0**
CPP	2·5	4·1 ± 0·8	71	0·0‡	0**
Clonidine	0·5	1·3 ± 0·6†	18**	17·9 ± 3·3‡	71
Repeated morphine injections					
Saline	—	5·4 ± 0·9	67	3·8 ± 1·4	33
(±)-Fenfluramine	5	3·6 ± 1·2	39	0·0‡	0*
CPP	2·5	3·7 ± 1·1	33	0·0‡	0*
Clonidine	0·5	0·8 ± 0·5‡	6**	12·7 ± 3·5†	53

At least 16 animals per group were used.
Drugs were administered 1 h before naloxone.
‡ $P < 0·01$ compared with saline (Student's *t*-test). **$P < 0·01$ compared with saline (χ^2 test).
† $P < 0·05$ compared with saline (Student's *t*-test). *$P < 0·05$ compared with saline (χ^2 test).

tion on touch, salivation and abnormal posture were observed occasionally. Animals presenting at least three wet dog shakes (shaking the whole body) were considered positive. Jumping consisted of the animals leaping onto the edge of the box with all four feet off the ground or occasionally jumping out of the box without clinging to the edge. After each jump the animal was immediately replaced in the box. The animals were considered positive if they made at least three jumps. The number of wet dog shakes and jumps per rat were also counted.

Differences between experimental groups and their controls were analysed statistically by the χ^2 test (proportions) or Student's *t*-test (numbers of wet dog shakes and jumps).

Drugs. (+)-Fenfluramine, CPP and clonidine were injected intraperitoneally 1 h before naloxone. Drugs were given at doses reported to act significantly on monoamine mechanisms. The appropriate references are given in brackets for each compound and dose: (+)-fenfluramine HCl (Servier, Neuilly, France) 5 mg kg^{-1} (Garattini et al 1975); CPP HCl (Aldrich-Europe, Beerse, Belgium) 2·5 mg kg^{-1} (Samanin et al 1979); clonidine HCl (Pierrel, Milan, Italy) 0·5 mg kg^{-1} (Andén et al 1970). All drugs were dissolved in 0·9% NaCl (saline). The controls received only saline.

Results and discussion

As shown in Table 1, a large percentage (about 70%) of animals that had received the two schedules of morphine treatment exhibited wet dog shakes whereas jumping was much less represented in these animals. (+)-Fenfluramine and CPP completely blocked jumping but failed to significantly affect either the proportion of animals showing wet dog shakes or the mean numbers of episodes in the two groups. In contrast, clonidine signficantly reduced both measures for wet dog shakes with no effect on the proportion of animals showing jumping. The mean number of jumps in the two experimental groups was raised significantly by clonidine treatment. This effect has been previously reported by Tseng et al (1975). We have previously shown that agents increasing 5-HT transmission block jumping of morphine abstinent rats with no effect on ptosis and diarrhoea (Samanin et al 1980; Cervo et al 1981).

The present results further show that 5-HT mechanisms are preferentially involved in the expression of jumping in morphine abstinent rats. In this respect agents increasing 5-HT transmission behave very differently from clonidine which blocks most withdrawal signs except jumping (Meyer & Sbarber 1976; Tseng et al 1975; Cervo et al 1981). Clonidine has been shown to block human opiate-withdrawal symptoms (Gold et al 1978). More recently, 5-hydroxytryptophan, a precursor of 5-HT, was found to have some beneficial effects in the abstinence syndrome of morphine-heroin addicts (Bellini et al 1982). It might be of interest to see whether (+)-fenfluramine and CPP reduce some effects of withdrawal in opiate addicts.

This work was partially supported by CNR Contract No. 82.02060.56.

REFERENCES

Andén, N.-E., Corrodi, H., Fuxe, K., Hökfelt, B., Hökfelt, T., Rydin, C., Svensson, T. (1970) Life Sci. 9, 1: 513–523

Bellini, C., Calvani, M., Fenelli, A., Le Pera, G., Pietrini, U., Sicuteri, F. (1982) Int. J. Clin. Pharmacol. Res. 2: 127–133

Bläsig, J., Herz, A., Reinhold, K., Zieglgänsberger, S. (1973) Psychopharmacologia 33: 19–38

Cervo, L., Rochat, C., Romandini, S., Samanin, R. (1981) Psychopharmacology 74: 271–274

Garattini, S., Buczko, W., Jori, A., Samanin, R. (1975) Postgrad. Med. J. 51 suppl. 1: 27–35

Gibson, R. D., Tingstad, J. E. (1970) J. Pharm. Sci. 59: 426–427

Gold, M. S., Redmond, D. E. Jr., Kleber, H. D. (1978) Lancet 2: 599–602

Lal, H., Numan, R. (1975) Life Sci. 18: 163–168

Meyer, D. R., Sbarber, S. B. (1976) Pharmacologist 18: 236

Samanin, R., Mennini, T., Ferraris, A., Bendotti, C., Borsini, F., Garattini, S. (1979) Naunyn-Schmiedeberg's Arch. Pharmacol. 308: 159–163

Samanin, R., Cervo, L., Rochat, C., Poggesi, E., Mennini, T. (1980) Life Sci. 27: 1141–1146

Tseng, L.-F., Loh, H. H., Wei, E. T. (1975) Eur. J. Pharmacol. 30: 93–99

Vetulani, J., Bednarczyk, B. (1977) J. Pharm. Pharmacol. 29: 567–569

Wei, E., Loh, H., Way, E. L. (1973) J. Pharmacol. Exp. Ther. 184: 398–403

J. Pharm. Pharmacol. 1984, 36: 70–72
Communicated July 4, 1983

The effects of ethanol dependence on drug responsiveness of mouse isolated vas deferens

SERGIO DE MORAES*, FRANCISCO RUY CAPAZ, *Department of Pharmacology, Instituto de Ciências Biomédicas, University of São Paulo, CEP, Brasil*

The effects of ethanol dependence on the responsiveness of the mouse vas deferens to noradrenaline (NA), carbachol, barium and calcium were studied. Ethanol dependence increases the maximum responses to NA and carbachol whereas responsiveness to barium remains unaltered. The concentration-effect curve to calcium was shifted to the left (3·0-fold at the EC50 level). It is concluded that in vas deferens isolated from ethanol-dependent mice the increased responsiveness to NA, carbachol and calcium is a consequence of an enhanced calcium entry through voltage-independent calcium channels, as it has been reported for brain tissue.

It has been proposed that ethanol dependence could involve adaptive alterations of neuronal membrane structure and physiological properties (Chin & Goldstein 1977a; Johnson et al 1979). The work presented here was an attempt to examine the drug responsiveness of vasa deferentia isolated from ethanol dependent mice. We reasoned that if ethanol increases calcium permeability, as a consequence of its action on the smooth muscle cell membranes, then a long-term administration of ethanol should enhance the responsiveness of the mouse vas deferens to drug stimulation.

Method

Swiss-Webster mice, 30 to 40 g, at the beginning of the experiments, were housed in groups of ten, in a well-ventilated room, kept at 22–24 °C and under a reversed 12-h light/dark cycle (light on at 12.00 noon). Dependence on ethanol was produced according to Goldstein (1972). Briefly, groups of 10 mice were transferred to a glass chamber (52 × 34 × 27 cm), with food and water freely available. An infusion pump delivered ethanol, at a rate of 30 mg min^{-1}, onto a filter paper wick in a flask. A continuously variable respirator pump delivered air through the flask into the chamber at a rate giving a nominal ethanol flow of 30 mg min^{-1}. At the start of the experiments all mice received a priming dose of ethanol (1·25 g kg^{-1} i.p.) and thereafter were exposed to ethanol vapour over five days. To all mice, including the control group, a daily injection of the alcohol-dehydrogenase inhibitor, pyrazole (1·0 mmol kg^{-1} i.p.) was given at 10.00 am. Groups of 10 control mice were also placed in the glass chamber over five days, with only air flowing through it. After five days of ethanol exposure the infusion pump was stopped and air allowed to flow through the chamber. Ethanol withdrawal was assessed using the single signal called 'convulsion on handling' and quantitatively evaluated using a previously reported scoring system of 1 to 4 (Goldstein 1972). Over 80% of the mice exposed to

Table 1. The effect of ethanol dependence on sensitivity and maximum response of the vas deferens of the mouse to noradrenaline, carbachol, barium and calcium.

	n[a]	EC50 × 10^{-6} M (95% C.I.)[b]	Ratio of EC50's[c]	Maximum response (± s.e.)[d]
Noradrenaline				
Control	6	16·8 (12·1–23·2)	—	1074·8 (115·9)
Ethanol	6	15·4 (10·9–21·9)	1·09	1447·3 (113·7)*
Carbachol				
Control	10	5·2 (3·2–7·1)	—	873·9 (49·8)
Ethanol	10	2·8 (0·8–4·7)	1·85	1106·4 (74·3)*
		EC50 × 10^{-3} M		
Barium				
Control	6	3·2 (1·6–5·6)	—	1382·3 (74·7)
Ethanol	6	2·2 (1·5–3·3)	1·45	1344·2 (95·2)
Calcium				
Control	8	14·5 (8·2–25·8)	—	538·3 (83·2)
Ethanol	8	4·8 (2·5–9·3)*	3·0	542·8 (60·4)

[a] Number of experiments.
[b] Geometric mean with 95% Confidence Intervals.
[c] EC50 control/EC50 ethanol.
[d] Mg of tension/10 mg of wet weight of tissue.
* Significantly different from control ($P < 0·01$).

* Correspondence.