J. Pharm. Pharmacol. 1986, 38: 283–287
Received August 15, 1985

The effects of peripherally administered monoaminergic drugs on ethanol diuresis in rats

L. A. POHORECKY

Center of Alcohol Studies, Rutgers University, Piscataway, NJ 08854, USA

The effect of peripherally administered drugs that modify monoaminergic function, on ethanol (2·0 g kg^{-1}, intragastrically)-induced changes in urine output has been examined in rats. The α-noradrenoceptor agonist, clonidine (0·05–0·15 mg kg^{-1}) produced marked urine output and potentiated slightly the diuretic effect of ethanol. The α-noradrenoceptor antagonist, phentolamine (1–5 mg kg^{-1}) dose-dependently decreased ethanol-induced diuresis. *p*-Chloroamphetamine (0·8–2·0 mg kg^{-1}) produced significant diuresis and potentiated the diuresis produced by ethanol. Methysergide (1·25, 2·5 mg kg^{-1}), a 5-hydroxytryptamine receptor antagonist, had no effect on urine output while it depressed the ethanol-induced increase in urine output. Apomorphine (0·8, 1·5 mg kg^{-1}), a dopamine receptor agonist, did not modify urine output in either control or ethanol-treated animals, while the dopamine receptor antagonist, pimozide (0·75–3·0 mg kg^{-1}), dose-dependently decreased ethanol-induced diuresis, but had no effect on urine output in control animals. Since our previous research indicates that the intraventricular administration of drugs that alter dopaminergic and 5-HT function does not alter ethanol-induced diuresis, the interaction of these types of agents with ethanol-induced diuresis in the present study suggests that the interaction was mediated peripherally.

Recently the diuretic action of ethanol in rats has been described (Pohorecky, 1985a, b). Urine output dose-dependently increased with ethanol from 0·75 to 2·5 kg^{-1} but decreased after doses of 4·0 g kg^{-1} or higher. As with other actions of ethanol there was development of tolerance to ethanol diuresis and it was dose-related (Pohorecky 1985a). The mechanism by which ethanol alters urine output is not known, but is believed to be due to a central action, most likely via an effect on vasopressin release (Van Dyke & Ames 1951). A number of neurotransmitters are believed to be involved in the regulation of vasopressin release (Sklar & Schrier 1983). The most consistent evidence indicates that central noradrenergic mechanisms play an inhibitory role in vasopressin release (Kimura et al 1981; Seybold et al 1981). Recent work from this laboratory indicates that the diuretic action of ethanol involves noradrenergic mechanisms in brain (Pohorecky & Packard 1986). Thus noradrenaline and clonidine, an α-adrenoceptor agonist, given intraventricularly, increased the ethanol diuresis while phentolamine, an α-adrenoceptor antagonist, decreased it. Furthermore, propranolol, a β-adrenoceptor agonist, decreased ethanol diuresis while isoprenaline, a β-adrenoceptor antagonist, increased it. Other neurotransmitters which have been implicated in the control of vasopressin release, such as dopamine and 5-HT administered intraventricularly, did not significantly alter the diuresis produced by ethanol.

Ethanol alters noradrenergic function in both central and peripheral neurons (Pohorecky 1974; Pohorecky & Jaffe 1975; Hunt & Majchrowicz 1980). The studies reported here were carried out to determine what contribution to ethanol's diuretic action could be expected from peripheral monoaminergic mechanisms.

METHODS

Male, Holtzman Sprague-Dawley rats, ca 250 g, were purchased from Charles River Breeding Laboratories, Inc. (Wilmington, MA). They were individually housed in metabolic cages (Wahmann Manufacturing Co., Timonium, MD), in a room that was temperature (21·0° ± 1·0 °C) and light (12:12 light : dark cycle, lights on at 0700h) controlled. Rats had free access to Purina food chow and water throughout, except as indicated. Animals were implanted with gastric cannulae, made of PE-100 (Clay Adams, Division of Becton Dickinson and Company, Parsippany, NJ) that were exteriorized in the scapular region. Four days after surgery, animals were handled daily for about 1 min to adapt them to human contact and the injection procedure.

One week after surgery animals were weighed and the patency of the cannulae was checked by injecting 1 ml of water. Animals were tested the next day beginning at noon (i.e. during the waking period). To achieve hydration, animals were injected intragastrically with a fluid load, corresponding to 25·0 ml kg^{-1} (Linkola et al 1978), consisting of water for the control group, and an appropriate dilution of

95% ethanol for the experimental group. Urine was then collected and measured at hourly intervals for 3 h. Food and water bottles were removed from the cages for the duration of the experiment. Ethanol (1·25 g kg^{-1}, 5% w/v, a dose that produces moderate diuresis allowing the detection of drug-induced potentiation or inhibition) or 0·9% NaCl (saline) was given intragastrically according to the following schedule with respect to the intraperitoneal drug treatment: apomorphine hydrochloride (Sigma Chemical Company, St. Louis, MO) 0·8 and 1·3 mg kg^{-1} prepared in 0·1% ascorbic acid, 10 min; clonidine hydrochloride (Boehringer Ingelheim Ltd., Ridgefield, CN) 0·05–0·1 mg kg^{-1}, 30 min; phentolamine hydrochloride (Ciba Geigy Corporation, Summit, NJ) 1–5 mg kg^{-1}, 30 min; pimozide (McNeil Laboratories, Inc., Fort Washington, PA) 0·75–3·0 mg kg^{-1}, solubilized in an acid solution and administered intragastrically, 30 min.

Breath ethanol concentration was determined at several points after treatment with ethanol using a method which allows repeated testing of subjects (Pohorecky & Brick 1982). The method consisted of quantifying ethanol present in a 1 ml sample of rebreathed air. Animals were allowed to breathe into a closed cylinder for 10 s. An air sample was taken from a needle port with an air tight syringe, and was injected directly into a gas chromatograph (Gow Mac Instruments Company, Madison, NJ). The chromatograph was equipped with a column (1/8 inch by 6 feet) packed with 50–80 mesh 'Porapak N'. Column temperature was 160 °C and the carrier gas flow (helium) was 30 ml min^{-1}.

Results were calculated and are presented as mean hourly urine volume ± s.e.m. and as the total urine volume for the 3 h experiment. A split plot design was used with ethanol as the plot variable and time of urine sampling as the subplot variable. Since ethanol and time are quantitative variables, they were analysed by regression analysis as part of the ANOVA (Bliss 1967; Cochran & Cox 1957). Because of the design of these experiments, the triple interaction (time × ethanol × drug) is of primary importance in the analysis of the data. Differences between means were considered statistically significant when $P \leq 0{\cdot}05$.

RESULTS

From Table 1 it is evident that clonidine had a significant effect on urine output in naive rats ($F(1,16) = 60{\cdot}46$; $P \leq 0{\cdot}001$). This effect was highly dependent on the time after treatment ($F(2,32) = 62{\cdot}00$; $P \leq 0{\cdot}001$), the diuresis being particularly great during the first collection period (Fig. 1A). It is possible that the gastric fluid load actually limited the full expression of diuresis in animals treated with the higher dose of clonidine. Ethanol treatment, as we had seen previously (Pohorecky 1985a), increased urine output primarily during the first post-treatment hour ($F(1,16) = 4{\cdot}47$; $P \leq 0{\cdot}05$), but at the dose used diuresis was much smaller than that produced by clonidine. When the two drugs were combined, urine output was only slightly higher than that seen in the group given clonidine alone. The triple interaction (time × ethanol × clonidine) was statistically significant ($F(2,32) = 4{\cdot}21$; $P \leq 0{\cdot}024$).

Table 1. Effect of pretreatment with drugs that modify monoaminergic function on ethanol (1·25 g kg^{-1}, 5% w/v solution) induced urine output in rats.

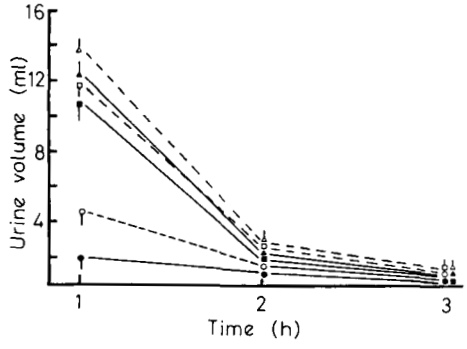
Treatment	(mg kg^{-1})	Total urine output (ml)	
Clonidine	0	3·67 ± 0·75	7·31 ± 0·86*
	0·05	13·11 ± 0·66*	15·33 ± 0·40*
	0·15	15·56 ± 1·26°	16·58 ± 0·44°¶
Phentolamine	0	3·03 ± 0·90	7·26 ± 0·54*
	5·0	3·82 ± 0·48	5·23 ±0·37°¶
	7·5	3·23 ± 0·23	2·85 ± 0·18°
	10·0	3·41 ± 0·57	1·97 ± 0·62°
Apomorphine	0	6·00 ± 0·22	8·76 ± 0·40*
	0·8	5·34 ± 0·35	6·94 ± 0·44
	1·5	5·74 ± 0·52	6·54 ± 0·56
Pimozide	0	4·33 ± 0·35	8·68 ± 0·57*
	0·75	4·07 ± 0·32	7·42 ± 0·79§
	1·50	4·18 ± 0·28	6·33 ± 0·67°§
	3·00	5·40 ± 0·09	3·80 ± 0·17°§
PCA	0	4·13 ± 0·51	6·20 ± 0·24*
	0·8	5·53 ± 0·26*	9·41 ± 0·59°#
	2·0	10·27 ± 0·71*	12·45 ± 1·21°#
Methysergide	0	3·75 ± 0·49	6·65 ± 0·30*
	1·25	5·64 ± 0·69	2·17 ± 0·30°#
	2·50	5·28 ± 0·51	2·70 ± 0·67°#

Data represent mean total urine output + s.e.m. for groups of 6 rats.
* $P \leq 0{\cdot}05$ compared with control animals.
° $P \leq 0{\cdot}05$ compared with ethanol-injected animals.
¶ $P \leq 0{\cdot}05$ compared with corresponding clonidine or phentolamine-injected animals.
§ $P \leq 0{\cdot}05$ compared with corresponding apomorphine or pimozide-injected animals.
\# $P \leq 0{\cdot}05$ compared with corresponding PCA or methysergide-injected animals.

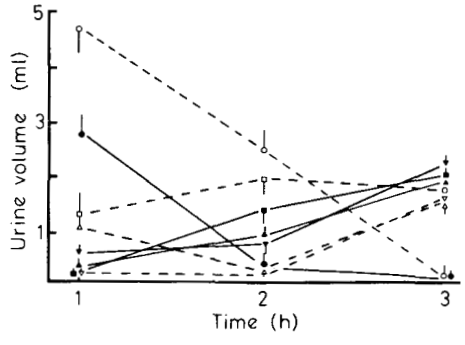
Phentolamine, on the other hand, initially depressed urine output followed by a rebound during the third collection period ($F(3,32) = 4{\cdot}84$; $P \leq 0{\cdot}01$) (Fig. 1B). However, the total urine output was not different from that of the control group. The elevated urine output produced by ethanol ($F(1,32) = 4{\cdot}57$; $P \leq 0{\cdot}05$) was significantly decreased by the drug ($F(6,64) = 5{\cdot}80$; $P \leq 0{\cdot}001$) and was dose-dependent (Table 1).

To modify 5-HT function, *p*-chloroamphetamine (PCA), which releases 5-HT, and methysergide, which blocks 5-HT receptors, were used (Table 1). The higher dose of PCA (2 mg kg^{-1}) increased urine volume by about 100% ($F(2,30) = 31{\cdot}93$; $P \leq 0{\cdot}001$) (Fig. 1C). The ethanol-induced diuresis ($F(1,30) = 21{\cdot}16$; $P \leq 0{\cdot}001$) was further potentiated by PCA treatment. The triple interaction of time × ethanol ×

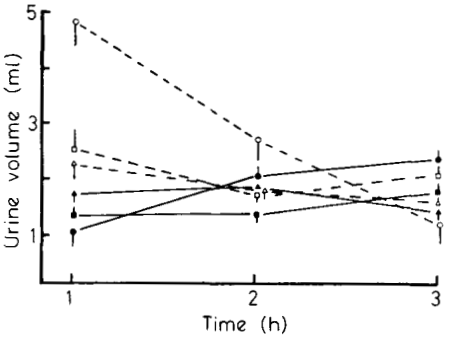
FIG. 1. Urine output of rats given an intragastric load of water (controls) of ethanol (ET = 1·25 g kg^{-1}) and pretreated intraperitoneally with:



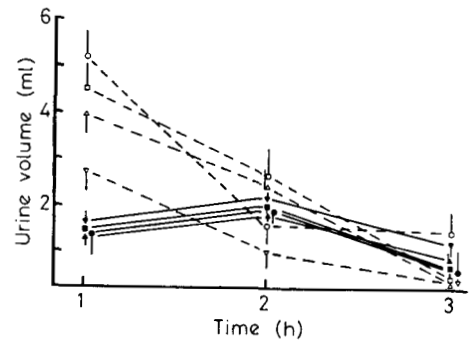
A. Clonidine (CL1 = 0·05; CL2 = 0·15 mg kg^{-1}) or saline (SA). Urine volume was measured hourly for 3 h. Results are expressed as the mean ± s.e.m. (vertical bars) for groups of 6 rats. Key: ● SA; ○ ET; ■ CL1; ▲ CL2; □ CL1 + ET; △ CL2 + ET.



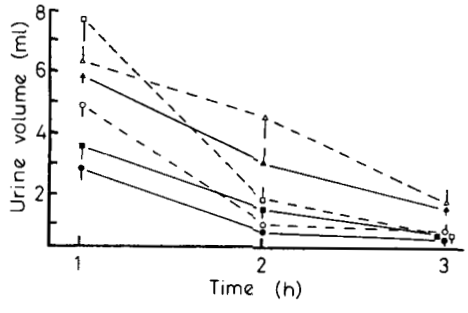
B. Phentolamine (PH1 = 5·0; PH2 = 7·5; PH3 = 10·0 mg kg^{-1}) or saline (SA). Urine volume was measured hourly for 3 h. Results are expressed as the mean ± s.e.m. (vertical bars) for groups of 6 rats. Key: ● SA; ○ ET; ■ PH1; ▲ PH2; ▼ PH3; □ PH1 + ET; △ PH2 + ET; ▽ PH3 + ET.



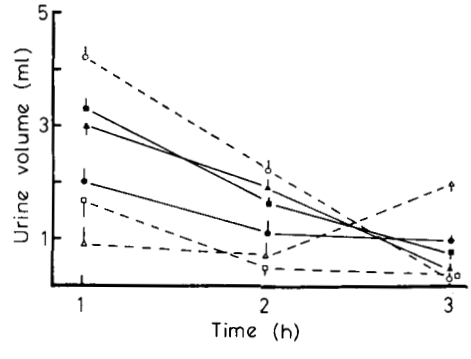
C. Apomorphine (AP1 = 0·8; AP2 = 1·5 mg kg^{-1}) or saline (SA). Urine volume was measured hourly for 3 h. Results are expressed as the mean ± s.e.m. (vertical bars) for groups of 6 rats. Key: ● SA; ○ ET; ■ AP1; ▲ AP2; □ AP1 + ET; △ AP2 + ET.



D. Pimozide (PI1 = 0·75; PI2 = 1·5; PI3 = 3·0 mg kg^{-1}) or saline (SA). Urine volume was measured hourly for 3 h. Results are expressed as the mean ± s.e.m. (vertical bars) for groups of 6 rats. Key: ● SA; ○ ET; ■ PI1; ▲ PI2; ▼ PI3; □ PI1 + ET; △ PI2 + ET; ▽ PI3 + ET.



E. PCA (PCA1 = 0·8; PCA2 = 2·0 mg kg^{-1}) or saline (SA). Urine volume was measured hourly for 3 h. Results are expressed as the mean ± s.e.m. (vertical bars) for groups of 6 rats. Key: ● SA; ○ ET; ■ PCA1; ▲ PCA2; □ PCA1 + ET; △ PCA2 + ET.



F. Methysergide (ME1 = 1·25; ME2 = 2·5 mg kg^{-1}) or saline (SA). Urine volume was measured hourly for 3 h. Results are expressed as the mean ± s.e.m. (vertical bars) for groups of 6 rats. Key: ● SA; ○ ET; ■ ME1; ▲ ME2; □ ME1 + ET; △ ME2 + ET.

PCA was highly significant ($F(4,60) = 6{\cdot}85$; $P \leq 0{\cdot}001$).

Methysergide had no effect on urine output in control rats. Although methysergide in the doses tested had no effect on urine output, the ethanol-induced diuresis ($F(1,30) = 5{\cdot}84$; $P \leq 0{\cdot}025$) was significantly decreased by methysergide treatment (Fig. 1D). Furthermore, the triple interaction was statistically significant ($F(4,60) = 2{\cdot}97$; $P \leq 0{\cdot}05$).

Turning to dopaminergic drugs, we examined the interaction of apomorphine, a dopamine receptor agonist, and of pimozide, a dopamine receptor antagonist, on ethanol-induced diuresis (Table 1). Apomorphine had no effect on urine output in control or ethanol-treated rats (Fig. 1E). Similarly pimozide had no significant effect on urine output in control rats (Fig. 1F). However the ethanol-induced elevated urine output ($F(1,32) = 23{\cdot}31$; $P \leq 0{\cdot}001$) was significantly and dose-dependently decreased by pimozide ($F(6,64) = 5{\cdot}83$; $P \leq 0{\cdot}001$).

One factor which should be considered when interpreting the results is whether blood ethanol levels were affected by the various drug pretreatments. This was evaluated in a separate series of experiments in which animals were treated with ethanol and the various drug pretreatments exactly as for the experiments where urine output was measured, except that breath samples for the estimation of blood ethanol levels were taken at 1 and 2 h after ethanol treatment. At the doses used, these drugs did not produce statistically significant changes in blood ethanol levels. Blood ethanol levels at 1 h after treatment were $115{\cdot}3 \pm 10{\cdot}1$ mg % and those at 2 h were $69{\cdot}9 \pm 9{\cdot}5$ mg %.

DISCUSSION

In the present experiments urine output was increased in the ethanol/clonidine group compared with that of the clonidine/saline group. Since urine output was so high in the clonidine group, the volume of the gastric load may have limited the expression of diuresis in the combined treatment groups. These results support our recent findings (Pohorecky 1985a) showing that ethanol increased urine output 163 and 154% in rats given 2·5 and 5·0 μg of clonidine, intraventricularly.

Clonidine is known to produce a dose-dependent diuresis upon peripheral and central administration (Reid et al 1984; Roman et al 1979; Miller 1980). Inhibition of vasopressin release is believed to be the primary mechanism for this drug-induced diuresis (Reid et al 1979). Research by others however indicates that clonidine may act primarily on the kidneys rather than by altering secretion of vasopressin. Investigators who concluded that clonidine acts via vasopressin release have generally used anaesthetized animals (dogs, rats), while researchers who did not find an involvement of vasopressin in clonidine diuresis, used unanaesthetized animals (rat, dog, isolated hypothalamic-pituitary system). Miller (1980) has proposed that the clonidine-produced acute elevation of blood pressure causes an increase in renal blood flow and a subsequent increase in glomerular filtration rate which gives rise to diuresis. Furthermore, the resulting decline in plasma volume and osmolality may stimulate vasopressin release which may overcome any inhibitory action of the drug in anaesthetized, volume-expanded animals. A similar mechanism may explain the small interaction between clonidine and ethanol with respect to diuresis in the present studies compared with those when clonidine was administered intraventricularly. When administered intraventricularly, clonidine would have little, if any, direct action on the kidneys, rather it would primarily inhibit vasopressin release. The stimulation of vasopressin release produced by renal effects of peripherally-injected clonidine in the present studies may be sufficiently strong to minimize the inhibitory effects of ethanol on vasopressin secretion. Thus, while clonidine (2·5 μg) given intraventricularly increased urine output by 1·5-fold, a 0·05 μg kg^{-1} dose given intraperitoneally elevated urine output 4·4 fold. However, urine output was increased 1·3-fold by ethanol in rats given the 2·5 μg dose of clonidine intraventricularly but only about 10·3% in rats given clonidine (0·05 mg kg^{-1}) intraperitoneally.

Phentolamine's effect on urine output, on the other hand, was independent of the route of administration. When administered intraventricularly or intraperitoneally, it blocked the diuretic action of ethanol. It is likely, therefore, that even with peripheral administration, the primary effect of phentolamine is on central noradrenergic mechanisms regulating vasopressin release. Of significance is that phentolamine by itself had no effect on urine volume, it nevertheless decreased urine output produced by ethanol. Phentolamine was found ineffective in suppressing plasma vasopressin increase produced by clonidine in volume-expanded anaesthetized animals (Reid et al 1979). Conversely, phenoxybenzamine, another α-adrenoceptor antagonist, decreased urine volume in non-volume expanded, non-anaesthetized animals (Miller 1980). The latter two findings support the suggestion, made above, that ethanol interacts

with clonidine primarily when this drug alters vasopressin secretion centrally and not via peripheral mechanisms.

Previously we have found no significant effect on ethanol diuresis of intraventricularly administered agents which modified 5-HT function. In this study the higher dose of peripherally administered PCA (2 mg kg^{-1}) increased urine output, confirming a previous report (Stein et al 1981). Furthermore, both doses of PCA further increased ethanol-induced diuresis. These results indicate that the interaction of ethanol with PCA probably results from the peripheral 5-HT action of this drug. The specificity of this effect was indicated by the fact that methysergide, a 5-HT receptor antagonist, inhibited ethanol diuresis.

Dopaminergic drugs, when given intraventricularly, had no effect on ethanol diuresis. Similarly, apomorphine, intraperitoneally, had no effect on urine output nor did it alter ethanol-induced diuresis. Urine output in rats treated with ethanol and pimozide, on the other hand, was significantly less compared with that of rats treated with ethanol or the higher dose of pimozide alone. Therefore it is likely that the effect of pimozide is exerted directly on the kidneys. The lack of effect of apomorphine has yet to be explained. It has been proposed that in addition to noradrenaline, dopamine may be a neurotransmitter in the kidneys (Dinerstein et al 1979; Morgunov & Baines 1981). Thus, peripherally administered dopamine has a strong diuretic effect (Deis & Alonso 1970; Wassermann et al 1980). On the other hand, it has also been proposed that the diuretic effect of dopamine is mediated via renal α-noradrenoceptors (Lehr et al 1967; Baggio & Ferrari 1981).

Ethanol alters monoaminergic function in both brain and peripheral tissues (Pohorecky 1974; Pohorecky & Jaffe 1975; Hunt & Majchrowicz 1980). The results presented here suggest that both central and peripheral monoaminergic mechanisms may be involved in the diuresis produced by ethanol. The reported direct renal effects of ethanol (Nicholson & Taylor 1938; Van Thiel et al 1977) may be mediated by local noradrenergic or dopaminergic mechanisms. Furthermore, the effect of ethanol on urine output in man can be modified in individuals receiving drug treatments that modify monoaminergic function.

Acknowledgements

Supported by USPHS grants AA 00045, 04238 and a Biomedical Research Support Grant from Rutgers University. I acknowledge the technical assistance provided by Mrs Eva Kasziba. I also thank the generous gift of drug samples provided by Boehringer Ingelheim, Ltd., Ciba Geigy Corporation and McNeil Laboratories.

REFERENCES

Baggio, G., Ferrari, F. (1981) Life Sci. 28: 1449–1456

Bliss, E. I. (1967) Statistics in Biology, Vol. I, pp 368–383

Cochran, W. G., Cox, G. M. (1957) Experimental Design, John Wiley and Sons, 293–316

Deis, R. P., Alonso, N. (1970) J. Endocrinol. 47: 129–130

Dinerstein, R. J., Vannice, J., Henderson, R. C., Roth, L. J., Goldberg, L. I., Hoffmann, P. C. (1979) Science 205: 497–499

Hunt, W. A., Majchrowicz, E. (1980) in: Majchrowicz, E., Noble, E. P. (eds) Biochemistry and Pharmacology of Alcohol, Plenum Press, New York, pp 167–183

Kimura, T., Share, L., Wang, B. C., Crofton, J. T. (1981) Endocrinology 108: 1829–1836

Lehr, D., Mallow, J., Krukowsky, M. (1967) J. Pharmacol. Exp. Ther. 158: 150–163

Linkola, J., Ylikahri, R., Fyhrquist, F., Wallenius, M. (1978) Acta Physiol. Scand. 104: 180–187

Miller, M. (1980) J. Pharmacol. Exp. Ther. 214: 608–613

Morgunov, N., Baines, A. D. (1981) Am. J. Physiol. 240: F75–F81

Nicholson, W. M., Taylor, H. M. (1938) J. Clin. Invest. 17: 279–285

Pohorecky, L. A. (1974) J. Pharmacol. Exp. Ther. 189: 380–391

Pohorecky, L. A. (1985a) J. Pharm. Pharmacol. 37: 271–273

Pohorecky, L. A. (1985b) Alcohol 2: 659–666

Pohorecky, L. A., Jaffe, L. (1975) Res. Comm. Chem. Pathol. Pharmacol. 12: 433–448

Pohorecky, L. A., Brick, J. (1982) Pharmacol. Biochem. Behav. 16: 693–696

Pohorecky, L. A., Packard, K. (1986) Alcoholism: Clin. Exp. Res. in press

Reid, I. A., Nolan, P. L., Wolf, J. A., Keil, L. C. (1979) Endocrinology 104: 1403

Reid, I. A., Ahn, J. N., Trinh, T., Shackelford, R., Weintraub, M., Keil, L. C. (1984) J. Pharmacol. Exp. Ther. 229: 1–8

Roman, R. J., Cowley, A. W., Jr., Lechene, C. (1979) Ibid 211: 385–393

Seybold, V., Elde, R., Hokfelt, T. (1981) Endocrinology 108: 1803–1808

Sklar, A. H., Schrier, R. W. (1983) Physiol. Rev. 63: 1242–1280

Stein, J. M., Wayner, M. J., Kantak, K. M. (1981) Pharmacol. Biochem. Behav. 15: 297–301

Van Dyke, H. B., Ames, R. G. (1951) Acta Endocrinol. 7: 110–121

Van Thiel, D. H., Gavaler, J. S., Little, J. M., Lester, R. (1977) Metabolism 26: 857–865

Wassermann, K., Huss, R., Kullmann, R. (1980) N.-S. Arch. Pharmacol. 312: 77–83