Sulpiride is clinically used as a drug with mixed antipsychotic and antidepressant effects[5]. There is one open-label study[26] and one double-blind study comparing sulpiride with amitriptyline[27] that documents sulpiride's antidepressant effects. Unfortunately, the investigators in both these studies did not note whether optically active or racemic drug was administered. Interestingly, a single case of mania caused by metoclopramide has been recently reported[28]. Since induction of mania is a phenomenon usually observed with antidepressants, this suggests that other substituted benzamides may also have antidepressant effects.

Because antidepressants demonstrated high affinity for the mesolimbic non-dopaminergic 3[H]-(−)-sulpiride sites described here, binding of sulpiride to these sites may mediate the antidepressant effects of this drug. In fact, binding of tricyclic and other antidepressants to such sites may be relevant for their therapeutic effects. This latter hypothesis is supported by the fact that IC 50 values obtained for the tricyclic antidepressants were consistent with their therapeutic serum levels[29]. Further studies are needed to associate these mesolimbic 3[H]-(−)-sulpiride sites with a particular neurotransmitter.

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3 Freedman, S.B., Mustafa, A.A., Poat, J.A., Senior, K.A., Wait, C.P., and Woodruff, G.N., Neuropharmacology *20* (1981) 1151.
4 Herrara-Marschitz, M., Stahle, L., Tossman, U., Zetterstrom, T., and Ungersteht, U., Acta psychiat. scand. *69*, suppl. 311 (1984) 147.
5 Jenner, P., and Marsden, C.D., in: Typical and Atypical Antidepressants, p. 85. Eds E. Costa and G. Racagni. Raven Press, New York 1982.
6 Zahniser, N.R., and Dubocovich, M.L., J. Pharmac. exp. Ther. *227* (1983) 592.
7 Burt, D.R., Creese, I., and Snyder, S.H., Molec. Pharmac. *12* (1976) 800.
8 Bradford, M.M., Analyt. Biochem. *72* (1976) 248.
9 Freedman, S.B., Poat, J.A., and Woodruff, G.N., Neuropharmacology *20* (1981) 1323.
10 Woodruff, G.N., and Freedman, S.B., Neuroscience *6* (1981) 407.
11 Memo, M., Govoni, S., Carboni, E., Trabucchi, M., and Spano, P.F., Pharmac. Res. Commun. *15* (1983) 191.
12 Mereu, G., Casu, M., and Gessa, G.L., Brain Res. *264* (1983) 105.
13 Seeman, P., Pharmac. Rev. *32* (1980) 229.
14 Theodorou, A.E., Hall, M.D., Jenner, P., and Marsden, C.D., J. Pharm. Pharmac. *32* (1980) 441.
15 Freedman, S.B., Poat, J.A., and Woodruff, G.N., J. Neurochem. *38* (1982) 1459.
16 Oki, K., and Nomura, Y., Devl Neurosci. *5* (1982) 379.
17 Theodorou, A.E., Jenner, P., and Marsden, C.D., Life Sci. *32* (1983) 1243.
18 Feighner, J.P., Allen, G.C., Fabre, L.F., Rickels, K., and Smith, W.T., J. Am. med. Ass. *249* (1983) 3057.
19 Clow, A., Jenner, P., Marsden, C.D., Reavill, C., and Theodorou, A.E., Br. J. Pharmac. (proc.) (1979) 433P.
20 Agnati, L.F., Cortelli, P., De Camillis, E., Benfenati, F., Orlandi, F., and Fresia, P., Neurosci. Lett. *15* (1979) 289.
21 Benfenati, F., Bernardi, P., Cortelli, P., Capelli, M., Adani, C., Calza, L., and Agnati, L.F., Neurosci. Lett. *26* (1981) 289.
22 Hoffman, M., Jommi, G.C., Montefusco, O., Tonon, G.C., Spano, P.F., and Trabucchi, M., J. Neurochem. *32* (1979) 1547.
23 Schmidt, M., Imbs, J.L., Giesen, E.M., and Schwartz, J., J. Cardiovasc. Pharmac. *5* (1983) 86.
24 Caldara, R., Masci, E., Cambielli, M., Tittobello, A., Piepoli, V., and Barbieri, C., Eur. J. clin. Pharmac. *25* (1983) 319.
25 Montanaro, N., Dall'Olio, R., Gandolfi, O., and Vaccheri, A., in: Typical and Atypical Antidepressants, p. 341. Eds E. Costa and G. Racagni. Raven Press, New York 1982.
26 Benkert, O., and Holsboer, F., Acta psychiat. scand. *69* suppl. 311 (1984) 43.
27 Standish-Barry, H.M.A.S., Bouras, N., Bridges, P.K., and Watson, J.P., Psychopharmacology *81* (1981) 258.
28 Ritchie, K.S., and Preskorn, S.H., J. clin. Psychiat. *45* (1984) 180.
29 Silverman, J.J., Brennan, P., and Friedel, R.O., Psychosomatics *20* (1979) 736.
30 Cheng, Y.C., and Prusoff, W.H., Biochem. Pharmac. *22* (1973) 3099.

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Lowering of liver acetaldehyde but not ethanol concentrations by pretreatment with taurine in ethanol-loaded rats

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Summary. A rise in blood and liver acetaldehyde concentrations following ethanol loading (1.5 g/kg b.wt) was significantly reduced when rats were pretreated orally with taurine (0.5 g/kg), a potent in vitro activator of yeast aldehyde dehydrogenase. This taurine pretreatment produced a 4-fold increase in liver taurine content.
Key words. Taurine; acetaldehyde; ethanol; ALDH.

We have reported that the oral administration of a clinical dose of pantethine results in a significant inhibition of blood acetaldehyde elevation following alcohol administration to nonflushing, but not to flushing subjects[1]. The pantethine action is based upon the interaction of hepatic aldehyde dehydrogenase and pantethine-related metabolites formed in the liver, such as taurine, D-pantetheine, coenzyme A and D-pantothanate. During these studies, we observed that among these metabolites taurine most potently activates yeast ALDH activity in vitro[2]. The present study was conducted to investigate whether or not an oral administration of taurine could directly diminish a rise in blood acetaldehyde levels following ethanol loading of rats.

Materials and methods. Male Sprague-Dawley rats, weighing 200–260 g each, were starved overnight. A 15% ethanol solution was administered intragastrically at a dose of 1.5 g/kg b.wt. Taurine (Sigma Chemical Co., St Louis) was administered intragastrically in the early morning 30 min before ethanol administration at a dose of 0.5 g/kg b.wt. Rats were killed by exsangui-

nation from the carotid artery 2 h after alcohol administration. A 0.15 ml sample of blood was added to 1.95 ml of ice-cold 1.06 M perchloric acid solution in an ice-cold test tube, which was tightly capped with a rubber stopper. Livers were removed and immediately frozen in liquid nitrogen. The frozen livers were pulverized in homogenizers cooled with liquid nitrogen. Ethanol and acetaldehyde concentrations in the blood and liver were determined according to our previously reported methods[3,4]. Average recovery using this method was found to be 100.1% for acetaldehyde and 98.1% for ethanol. Plasma and liver amino acid contents were determined 1 h after taurine administration. Quantitative determinations of serum and liver amino acids were carried out with a Nihon-Denshi JCL-6AL amino acid analyzer as described previously[5]. Results were expressed as mean ± SD. Significance ($p < 0.05$) of deviation from the control was calculated by Student's t-test.

Results and discussion. Serum and liver taurine concentrations 1 h after the oral administration of taurine (0.5 g/kg b.wt) were much higher than in control rats: 11.2 ($p < 0.1$) and 4.0 ($p < 0.05$) times higher, respectively (table 1). Blood and liver acetaldehyde concentrations following ethanol loading were significantly reduced when rats were pretreated with taurine. Taurine, an amino acid which does not occur in proteins, exists free in many cells in large amounts. In chronic alcoholics, urinary taurine excretion increases and plasma taurine levels diminish[6]. The effect of taurine administration on ethanol-induced sleeping time in mice[7] and on alcohol withdrawal[8] have been studied. The former study revealed that the brain-depressant effect of ethanol (4 g/kg b.wt) was markedly reduced in mice by simultaneous i.p. injection of taurine (45 mg/kg b.wt). The latter study stated that an oral administration of taurine (3 g/day) for 7 days showed preventive and therapeutic effects on alcohol withdrawal. Blood acetaldehyde levels after drinking a taurine-containing alcoholic beverage were much lower than those after drinking alcohol (A. Watanabe et al., unpublished observations). Taurine as well as pantethine may be clinically useful for preventing alcoholic liver damage and alcohol addiction, since acetaldehyde has been implicated in the pathogenesis of these problems[9].

Table 1. Effect of intragastric taurine administration on serum and liver taurine concentrations

	Serum (μmoles/l)	Liver (μmoles/kg)
Control	253 ± 15	2030 ± 493
Taurine	2833 ± 1305*	7933 ± 1436**

Mean ± SD. No. of rats = 3. *$p < 0.1$ and **$p < 0.05$.

Table 2. Effect of intragastric taurine administration on ethanol and acetaldehyde concentrations in the blood and liver following ethanol loading

	Blood Ethanol (mM)	Acetaldehyde (μm)	Liver Ethanol (mmoles/kg)	Acetaldehyde (μmoles/kg)
Control	25.9 ± 2.2	3.9 ± 0.7	15.5 ± 1.5	13.5 ± 3.2
Taurine	24.1 ± 5.0	1.5 ± 0.6*	19.0 ± 1.5	4.4 ± 0.5*

Mean ± SD. No. of rats = 3. *$p < 0.05$.

1 Watanabe, A., Hobara, N., Kobayashi, M., Nakatsukasa, H., and Nagashima, H., Alcoholism Clin. exp. Res. in press (1985).
2 Watanabe, A., Hobara, N., and Nagashima, H., Pharmacology, in press (1985).
3 Hobara, N., Watanabe, A., Kobayashi, M., Nakatsukasa, H., Nagashima, H., Fukuda, T., and Araki, Y., Jap. J. clin. Pharmac. Ther., in press (1985).
4 Watanabe, A., Hobara, N., Nakatsukasa, H., Shiota, T., Kobayashi, M., and Nagashima, H., Res. exp Med. *185* (1985) 13.
5 Watanabe, A., Kobayashi, M., Hobara, N., Nakatsukasa, H., Nagashima, H., and Fujimoto, A., Alcoholism Clin. exp. Res. *9* (1985) 14.
6 Turner, P.P., and Brun, V.C., J. Surg. Res. *4* (1964) 423.
7 Iida, S., and Hikichi, M., J. Stud. Alcohol. *37* (1976) 19.
8 Ikeda, H., Suwaki, H., and Horii, S., Psychiat. Med. *20* (1978) 912.
9 Lieber, C.S., Gastroenterology *79* (1980) 373.

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Synthesis of trypsin inhibitor CMTI III from squash seeds *(Cucurbita maxima)*[1]

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Summary. Using the solid-phase procedure, a 29-peptide cross-linked by three disulphide bridges was synthesized. The synthetic product was shown to be identical with the trypsin inhibitor CMTI III from squash seeds *(Cucurbita maxima)*.
Key words. 29-peptide; trypsin inhibitor; solid phase peptide synthesis; squash seeds.

In 1980 Polanowski et al.[2] isolated low molecular mass (M_r 3300) polypeptide trypsin inhibitors, coded CMTI I and CMTI III[3], from squash seeds *(Cucurbita maxima)*. The inhibitors were purified with immobilized trypsin[4]. In 1981 Nowak et al.[5] published one sequence of the CMTI III. The authors suggest that the inhibitor molecules consists of 28 amino acid residues cross-linked by two disulphide bridges.

In 1983 another sequence of CMTI III and a sequence of CMTI I were published by Wilusz et al.[6]. According to these authors, the molecule of CMTI III, as well as that of CMTI I consist of 29 amino acid residues cross-linked by three disulphide bridges. The sequences of the two inhibitors differ in only one position, Lys_9[7] in CMTI III being replaced by Glu_9 in CMTI I[6]. It was established that Arg_5-Ile_6 is the reactive site peptide bond in both inhibitors[5,6]. The interaction of CMTI III or CMTI I with trypsin is accompanied by cleavage of this peptide bond, resulting in the modified inhibitors CMTI III* and CMTI I*[2,4], the molecules of which consist of two peptide chains held together by one disulphide bridge[5].

In this paper we present the synthesis of two polypeptides: the 28-peptide with the sequence suggested for CMTI III by Nowak et al.[5]: Arg-Val-Cys-Pro-Arg-Ile-Leu-Met-Lys-Cys^{19}-Lys-Lys-Asp-Gln-Ser-Asp-Leu-Ala-Glu-Val^{20}-Cys-His-Leu-Glu-Cys-Gly-Gly-Tyr^{28}, and the 29-peptide with the sequence suggested