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# Modulation of seizure pattern in the rat by neutral metabolites of indoleamines

AKEMI MINEGISHI, RYUJI FUKUMORI, TETSUO SATOH*, HARUO KITAGAWA, *Department of Biochemical Pharmacology, Faculty of Pharmaceutical Sciences, Chiba University, Yayoi-cho 1–33, Chiba 260, Japan.*

The neutral metabolites of indoleamines (biogenic alcohols such as tryptophol and 5-hydroxytryptophol, and biogenic aldehydes such as indoleacetaldehyde and 5-hydroxyindoleacetaldehyde) have been reported to be biologically active, i.e., to have sedative (Minegishi et al 1979), sleep-inducing (Sabelli et al 1969; Taborsky 1971) and anticonvulsant properties (Fukumori et al 1980a). Our recent finding that tryptophol and/or its metabolite indoleacetaldehyde decreased seizure susceptibility in mice suggested the involvement of neutral metabolites of indoleamines in the regulation of seizure threshold (Satoh et al 1979a). On the other hand, it is well-established that the threshold for convulsion is modulated by a central aminergic system (Kilian & Frey 1973). In the present work, we examined the effect of centrally administered tryptophol on the components of leptazol (pentetrazol)-induced seizures in normal and reserpinized rats, in the belief that neutral metabolites of indoleamines may be involved in the modulation of the seizure pattern by the 5-HT-ergic system.

*Materials and methods*

Male Wistar rats, 180–220 g, were fitted with indwelling cannulae at least 1 week before the experiments, as described by Satoh et al (1979b). Tryptophol (1 $\mu$mol) was dissolved in 8 $\mu$l of 0·9% w/v NaCl (saline) solution containing 25% ethanol and injected intracerebroventricularly (i.c.v.) Control animals received equivolume injections of the vehicle alone. The vehicle had no effect on leptazol-induced seizure. Leptazol was dissolved in saline solution and injected intraperitoneally into normal animals at a dose of 100 mg kg$^{-1}$ and into reserpinized animals at 70 mg kg$^{-1}$, 1 min after the i.c.v. injection of tryptophol or the vehicle. Reserpine (2 mg kg$^{-1}$, i.p.) was dissolved in 0·3 M sucrose with a few drops of glacial acetic acid. After the leptazol challenge, latencies were recorded to the onset of (1) clonic seizure with loss of righting reflex; and (2) tonic seizure with hindlimb extension. The seizure latency time was estimated as the time interval between the first sign of clonic seizure and the tonic seizure, according to Weiss et al (1960).

*Results*

Fig. 1 shows the effects of tryptophol on the latencies to the onset of the clonic and tonic seizures, and the seizure latency time in normal animals after leptazol administration. Tryptophol neither prolonged nor shortened the latency to clonic seizure, but it significantly prolonged the latency to tonic seizure and the seizure latency time (35 and 94% increases, respectively). Fig. 2 shows the effects of tryptophol in reserpinized animals. Treatment with reserpine potentiates the convulsant properties of leptazol and shortens the seizure latency time (Chen et al 1954). Thus, the dose of leptazol was reduced to 70 mg kg$^{-1}$ for better evaluation of the tryptophol effect. The latency to the onset of clonic seizure was also unaffected by i.c.v. injection of tryptophol in reserpinized rats. However, both the latency to tonic seizure and the seizure latency time were dramatically prolonged by tryptophol (164 and 458% increases, respectively) in reserpinized animals.

*Discussion*

Biogenic amines such as indole and catecholamines are deaminated by monoamine oxidase to the corresponding aldehydes (Udenfriend et al 1956). The intermediate metabolites, biogenic aldehydes, are further catabolized by either an oxidative (Erwin & Deitrich 1966) or a reductive (Tabakoff & Erwin 1970) pathway. Aldehyde reductase catalyses not only the reductive pathway in biogenic aldehyde metabolism but also the oxidation of exogenous alcohols (Satoh et al 1979a). Recently, we suggested that the manifestation of the anticonvulsant effect of tryptophol requires the conversion of tryptophol to its active metabolite, indoleacetaldehyde (Satoh et al 1979a). This view was confirmed by the finding that pretreatment with aldehyde reductase inhibitors such as diphenylhydantoin and chlorpromazine antagonized the anticonvulsant action of tryptophol. Of course, the pharmacological activity of exogenously administered tryptophol does not necessarily imply that endogenous products of indoleamines play a physiological role in the modulation of seizure threshold. However, other reports support the view that biogenic aldehydes and alcohols are partly responsible for the regulation of the threshold of convulsion. Ris et al (1975) observed that many conventional anticonvulsant drugs, including barbiturates, hydantoins and glutarimides, inhibit the brain aldehyde reductase activity, and suggested the involvement of deaminated metabolites of biogenic amines in the anticonvulsant action of these drugs. In addition, Bhattacharya & Sanyal (1978) reported that the threshold for leptazol seizure was reduced by prostaglandin $E_1$,

* Correspondence.

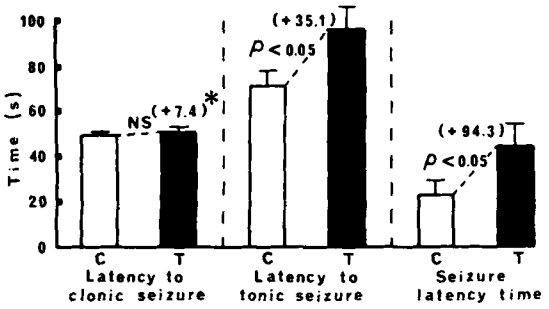


FIG. 1. Effect of tryptophol on the leptazol-induced seizure in normal rats. Leptazol (100 mg $kg^{-1}$ i.p.) was injected 1 min after tryptophol (1 μmole/animal i.c.v.) (■ T) or the vehicle (□ C). *Each column represents the mean of 10 animals. Vertical lines show s.e.m. Values in parentheses represent percent changes from the corresponding control values. *P*, level of significance of the difference from the control (Student's *t*-test). NS, Not significant.

which increases the 5-HT turnover and elevates the steady state concentrations of its neutral metabolites in the brain (Haubrich et al 1973; Fukumori et al 1980b).

In the present study, we have demonstrated that i.c.v. injection of tryptophol prolongs the latency to tonic seizure and the seizure latency time without affecting the latency to the onset of clonic seizure, and that the effect of tryptophol on the tonic component of seizure was more remarkable in reserpinized animals, in which the threshold for tonic seizure was reduced. These results suggest that the anticonvulsant effect of tryptophol may be manifested primarily by modulating the tonic component of seizure.

Since Chen et al (1954) demonstrated the ability of reserpine to potentiate the tonic component of seizure, many investigators have tried to elucidate the relationship between the threshold for convulsion and the central indoleamines and catecholamines. Lessin & Parks (1959) suggested that the reduction of the leptazol seizure threshold by reserpine is related to a decrease in the brain 5-HT concentration. They observed that subsequent administration of 5-hydroxytryptophan, a precursor of 5-HT, antagonized the action of reserpine. In addition, Kilian & Frey (1973) suggested that the threshold for the tonic component of the leptazol-induced seizure is modulated by the central 5-HT-ergic system. They observed that the threshold for the tonic extensor component of the seizure was elevated by 5-hydroxytryptophan, and conversely was lowered by *p*-chlorophenylalanine, an inhibitor of 5-HT synthesis. When analysing these data, however, it must be remembered that administration of the precursor increases not only the concentration of the amine but also that of its active metabolites, including 5-hydroxytryptophol and 5-hydroxyindoleacetaldehyde, in the brain. Thus, one may consider the possibility that deaminated metabolites of 5-HT may be involved, at least in part, in the action of the amine precursor. Our results indicate that the action of tryptophol on leptazol seizure closely resembles that of the 5-HT precursor. On the basis of the previous and present results, it is reasonable to conclude that neutral metabolites of indoleamines, such as biogenic aldehydes and alcohols, are implicated in the modulation of the seizure pattern by the 5-HT-ergic system.

There is clinical evidence that human epilepsy is causally related to an abnormality of brain indoleamine metabolism, and that anticonvulsant drugs modify indoleamine metabolism in epileptic patients. Measurement of indoleamine metabolites in the cerebrospinal fluid (c.s.f.) is the most commonly used method for studying their parent amine metabolism in the human central nervous system (Garelis & Sourkes 1974; Young et al 1980). Garelis & Sourkes (1974) found lower concentrations of c.s.f. 5-hydroxyindoleacetic acid (5-HIAA) in their epileptic patients, compared with control values. Chadwick et al (1975a) reported increased c.s.f. concentrations of 5-HIAA in anticonvulsant-treated epileptic patients. The rise in c.s.f. 5-HIAA was related to the number of anticonvulsants administered and the serum concentrations of phenobarbitone and diphenylhydantoin (Chadwick et al 1977). In another epileptiform disorder, myoclonus, Chadwick et al (1975b) have also shown that the

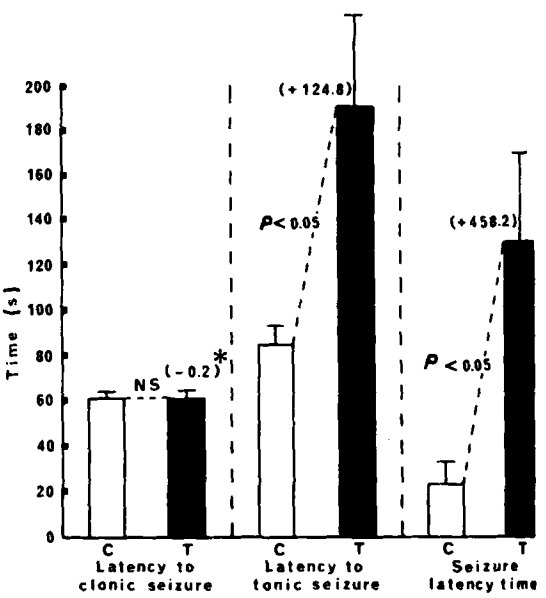


FIG. 2. Effect of tryptophol on the leptazol-induced seizure in reserpinized rats. Reserpine (2 mg $kg^{-1}$ i.p.) was injected 18 h before leptazol. Leptazol (70 mg $kg^{-1}$ i.p.) was injected 1 min after tryptophol (■ T) or the vehicle (□ C). Each column represents the mean of 9 animals. For details, see the legend to Fig. 1.

abnormally low c.s.f. 5-HIAA values were elevated in a dose-dependent manner after clonazepam therapy.

The significance of our observations in relation to the etiology and treatment of human epilepsy remains to be elucidated.

REFERENCES


Bhattacharya, S. K., Sanyal, A. K. (1978) Psychopharmacology 56: 235–237

Chadwick, D., Jenner, P., Reynolds, E. H. (1975a) Lancet 1: 473–476

Chadwick, D., Harris, R., Jenner, P., Reynolds, E. H., Marsden, C. D. (1975b) Ibid. 2: 434–435

Chadwick, D., Jenner, P., Reynolds, E. H. (1977) Ann. Neurol. 1: 218–224

Chen, G., Ensor, C. R., Bohner, B. (1954) Proc. Soc. Exp. Biol. Med. 86: 507–510

Erwin, V. G., Deitrich, R. A. (1966) J. Biol. Chem. 241: 3533–3539

Fukumori, R., Minegishi, A., Satoh, T., Kitagawa, H., Yanaura, S. (1980a) Psychopharmacology 69: 243–246

Fukumori, R., Minegishi, A., Satoh, T., Kitagawa, H., Yanaura, S. (1980b) Brain Res. 181: 241–244

Garelis, E., Sourkes, T. L. (1974) J. Neurol. Neurosurg. Psychiat. 36: 704–710

Haubrich, D. R., Perez-Cruet, J., Reid, W. D. (1973) Br. J. Pharmacol. 48: 80–87

Kilian, M., Frey, H.-H. (1973) Neuropharmacology 12: 681–692

Lessin, A. W., Parks, M. W. (1959) Br. J. Pharmacol. 14: 108–111

Minegishi, A., Fukumori, R., Satoh, T., Kitagawa, H., Yanaura, S. (1979) Res. Commun. Chem. Pathol. Pharmacol. 24: 273–287

Ris, M. M., Deitrich, R. A., von Wartburg, J. P. (1975) Biochem. Pharmacol. 24: 1865–1869

Sabelli, H. C., Giardina, W. J., Alivisatos, S. G. A., Seth, P. K., Ungar, F. (1969) Nature (London) 223: 73–74

Satoh, T., Fukumori, R., Nakagawa, I., Minegishi, A., Kitagawa, H., Yanaura, S. (1979a) Life Sci. 24: 2031–2036

Satoh, T., Fukumori, R., Minegishi, A., Kitagawa, H., Yanaura, S. (1979b) Res. Commun. Chem. Pathol. Pharmacol. 23: 297–311

Tabakoff, B., Erwin, V. G. (1970) J. Biol. Chem. 245: 3263–3268

Taborsky, R. G. (1971) Experientia 27: 929–930

Udenfriend, S., Titus, E., Weissbach, H., Peterson, R. E. (1956) J. Biol. Chem. 219: 335–344

Weiss, L. R., Nelson, J. W., Tye, A. (1960) J. Am. Pharm. Assoc. 49: 514–517

Young, S. N., Anderson, G. M., Gauthier, S., Purdy, W. C. (1980) J. Neurochem. 34: 1087–1092


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# $\alpha_2$-Adrenoceptors modulating diarrhoea in morphine-dependent rats

TOSHIO NAKAKI*, PAUL C. J. CHANG, YUKIKO TOKUNAGA, RYUICHI KATO, *Department of Pharmacology, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan*

Clonidine inhibits some naloxone-precipitated abstinence signs such as body shakes (Tseng et al 1975: Fielding et al 1978) and diarrhoea (Sparber & Meyer 1978) in several species, and nalorphine-elicited shaking behaviour in morphine-dependent rats (Vetulani & Bednarczyk 1977). The mechanisms by which clonidine reduced the morphine-abstinence syndrome are unknown. Atlas & Sabol (1980) suggested a possible close structural relationship between the ligand binding sites of $\alpha_2$-adrenoceptors and opiate receptors. The inhibitory effects of clonidine on abstinence signs, however, were not antagonized by a narcotic antagonist naloxone (Tseng et al 1975; Sparber & Meyer 1978) or nalorphine (Vetulani & Bednarczyk 1977), which suggests a mechanism of action unlike that of narcotics. The pharmacological effects of clonidine are most commonly interpreted as reflecting an effect on $\alpha_2$-adrenoceptors (Berthelsen & Pettinger 1977). There have been no reports which show directly in vivo that the inhibitory effects of clonidine on naloxone-precipitated abstinence signs are related to specific receptors. Therefore, we investigated effects of α-adrenoceptor agents to further characterize the type of receptors involved in the inhibitory effects of clonidine on naloxone-precipitated diarrhoea.

Male Wistar rats, 250–350 g, were cannulated into the right jugular vein under light ether anaesthesia and were subjected to continuous intravenous infusion of morphine or 0·9% NaCl (saline) at a rate of 4 mg $kg^{-1}$ $h^{-1}$ for 48 h as described previously (Nakaki et al 1980) using a modification of the method of Cox et al (1968). Immediately after the cessation of infusion, an α-adrenoceptor agonist or saline was injected subcutaneously. Naloxone (5 mg $kg^{-1}$ s.c.) with or without an α-adrenoceptor antagonist (s.c.) was injected 30 min after the cessation of infusion. Diarrhoea was observed for 4 h after the naloxone challenge. Diarrhoea was defined as: expulsion of soft wet faeces which did not possess or retain a pellet shape. The statistical analyses were done by Fisher's exact test (Rimm et al 1980).

The following drugs were used: morphine HCl (Sankyo Co. Ltd., Tokyo, Japan), naloxone HCl (Endo

* Correspondence.