J. Pharm. Pharmac., 1967, **19**, 170-175 Received October 10, 1966

The mechanism of the tranquillizing action of asarone from *Acorus calamus* Linn.

M. K. MENON AND P. C. DANDIYA

Asarone, the active principle of the indigenous Indian plant *Acorus calamus* Linn., did not cause any change in the noradrenaline content of whole brain of rats. Unlike tetrabenazine, pretreatment with asarone failed to block the effect of reserpine on the spontaneous motor activity and ptosis of mice, as well as the conditioned avoidance response of trained rats. These findings show that, though in chemical structure asarone resembles a part of the reserpine molecule, its mechanism of action is different from this drug. In animals in which brain noradrenaline has been lowered by pretreatment with α-methyl-L-tyrosine, the effects of asarone,namely, hypothermia, potentiation of barbiturate hypnosis and specific blockade of conditioned avoidance response, were found to be markedly enhanced. It seems that the sedative effect of asarone is dependent on the depression of the ergotropic division of the hypothalamus.

ASARONE, the active principle from the Indian plant *Acorus calamus* Linn., has tranquillizing activity and is similar in some of its properties to reserpine and chlorpromazine (Dandiya & Sharma, 1962; Dandiya & Menon, 1963, 1964, 1965). However, an important difference between the actions of asarone and reserpine is the inability of asarone to deplete brain 5-hydroxytryptamine (Dandiya & Menon, 1963).

Since the chemical structure of asarone as elucidated by Baxter, Dandiya, Kandel, Okany & Walker (1960) (i.e. *cis* isomer of 2,4,5-trimethoxy-1-propenylbenzene) resembles a moiety of the reserpine molecule we have investigated whether reserpine and asarone produce their tranquillizing effects by acting on the same receptors.

In animals treated repeatedly with α-methyltyrosine, the tyrosine hydroxylase inhibitor (Nagatsu, Levitt & Udenfriend, 1964) which lowers tissue noradrenaline content (Spector, Sjoerdsma & Udenfriend, 1965), we found that the central actions of both reserpine and chlorpromazine were markedly enhanced. Since the pharmacological actions of asarone are in some ways comparable to those of reserpine and chlorpromazine we also examined the effect of asarone in animals treated with α-methyl-L-tyrosine.

Experimental

A solution of asarone for injection was prepared by dissolving it in a few drops of absolute ethanol and adding warm 3% polysorbate 80 to the required volume. α-Methyl-L-tyrosine was dissolved in the minimum quantity of 3N sodium hydroxide and the pH adjusted to 8 using 0·1N hydrochloric acid. Rats or mice were used according to which species was the more suitable for a particular experiment. All the injections were made intraperitoneally.

The albino mice used were of either sex and weighed between 20–25 g. They were of Hindustan Antibiotics strain.

From the Department of Pharmacology, S.M.S. Medical College, Jaipur, India.

Noradrenaline content of whole brain of rats. Albino rats (CDRI strain) of either sex weighing between 100 and 150 g were given asarone (3 mg/kg) and after 45 min the animals were decapitated and the noradrenaline content of whole brain was estimated according to the method of Shore & Olin (1958). Animals treated with the solvent served as controls.

Effect of reserpine on the spontaneous motor activity of mice pretreated with asarone. A group of 20 mice was divided into 4 groups of 5 animals each. The first group was treated with reserpine (1 mg/kg). The second group was pretreated with asarone (3 mg/kg) followed 30 min later by reserpine (1 mg/kg). Since pretreatment with tetrabenazine prevents the action of reserpine (Quinn, Shore & Brodie, 1959), this drug was administered in a dose of 60 mg/kg to the third group of mice. 30 min later the animals were given reserpine (1 mg/kg). Solvent-treated animals served as controls. The four groups were observed for 6 hr and again 18–20 hr later, and compared. The degree of locomotor activity of groups of animals, their response to tactile stimulus, as well as the extent of ptosis in individual animals were scored and compared with those of the controls.

Effect of reserpine on the conditioned avoidance response of rats pretreated with asarone. Male albino rats (Haffkine strain) weighing between 100 and 125 g were trained to develop a conditioned avoidance response and an escape response. The technique is a minor modification of that of Cook & Weidley (1957) and is described in a previous communication (Dandiya & Sharma, 1962). Experiments were made between 9.00 and 12.00 hr at a room temperature of 24 $\pm 1°$.

Three groups of 10 trained rats each were treated with asarone (3 mg/kg), reserpine (1 mg/kg), and tetrabenazine (80 mg/kg) respectively. Another two groups of trained rats were pretreated with either asarone (3 mg/kg) or tetrabenazine (80 mg/kg), both were then given reserpine (1 mg/kg) 30 min later. 24 hr later the animals were examined for changes in their trained responses.

Effect of asarone on the rectal temperature of α-methyltyrosine-treated mice. Four groups of mice of 10 animals each were used. One group was treated with the solvent, another with asarone (1 mg/kg), a third with α-methyltyrosine followed by the solvent, and the fourth with α-methyltyrosine followed by asarone (1 mg/kg). α-Methyltyrosine was administered in three doses of 80 mg/kg each, the first dose was given 24 hr before, the second dose 18 hr before, the third dose 4 hr before either asarone or the solvent. Rectal temperature was recorded electronically in all the groups of mice immediately before the administration of asarone or solvent. The temperature recordings were again made 30 min after asarone or solvent administration, then at 1 hr intervals for the next 4 hr and again 16 hr later.

Effect of asarone on pentobarbitone-induced hypnosis of α-methyltyrosine-treated mice. The method adopted was similar to that of Dandiya & Cullumbine (1959). Groups of mice were treated with three doses of α-methyltyrosine according to the schedule mentioned above and 4 hr

after the last dose either the solvent or asarone (3 mg/kg) was given, 45 min later pentobarbitone sodium (40 mg/kg) was given.

Effect of asarone on the conditioned avoidance response of α-methyltyrosine-treated rats. Groups of rats trained for conditioned avoidance and escape responses were treated with α-methyltyrosine as mentioned previously. Asarone (3 mg/kg) was given 4 hr after the last dose of α-methyltyrosine. The animals were then examined for the loss of conditioned avoidance or escape responses at 1 hr intervals for 4 hr and then 24 hr later. The effect was compared with those obtained in control animals treated only with asarone (3 mg/kg) and also with rats receiving only α-methyltyrosine.

Results

Effect of asarone on the noradrenaline content of whole brain of rats. Asarone did not alter the noradrenaline content of whole brain of rats. The mean value (±s.e.) obtained in 6 solvent-treated animals was 0·508 ±0·088, and this was similar to that from 6 asarone-treated animals (0·521 ±0·090).

Effect of reserpine on the spontaneous motor activity and ptosis of mice pretreated with either asarone or tetrabenazine. Mice treated with either asarone or tetrabenazine were found to be sedated as seen by the reduced spontaneous activity and the degree of ptosis. Asarone caused a greater degree of sedation than tetrabenazine. The effect of these drugs lasted for 4–6 hr. The intensity of sedation observed in reserpine-treated animals was comparable to that of asarone, but the effect could be observed after 20 hr. When observed 18–20 hr after treatment with reserpine, animals which received asarone followed by reserpine showed an intensity of sedation and ptosis similar to the group treated with reserpine only. On the contrary, in animals treated with tetrabenazine and then reserpine, neither sedation nor ptosis was observed and responses resembled those of the solvent-treated controls. Thus whereas tetrabenazine blocked the effect of reserpine, asarone was ineffective.

Effect of reserpine on the conditioned avoidance response or escape response of rats pretreated with either asarone or tetrabenazine. The results are given in Table 1. The effect of asarone or tetrabenazine on

TABLE 1. EFFECT OF PRETREATMENT WITH ASARONE OR TETRABENAZINE ON THE CONDITIONED AVOIDANCE RESPONSE (CAR) AND ESCAPE RESPONSE (ER) BLOCKING EFFECT OF RESERPINE

Drug	No. of trained rats	20 hr after drug No. of animals showing blockade of		No. animals showing drug effect/total no. animals employed
		CAR	ER	
Control (untreated)	30	0	0	—
Reserpine	10	6	1	7/10
Asarone	10	0	0	0/10
Tetrabenazine	10	0	0	0/10
Asarone + reserpine	10	6	2	8/10
Tetrabenazine + reserpine ..	10	1	0	1/10

the conditioned avoidance response of trained rats did not persist for more than 4 hr and was not observed when the animals were examined 18–20 hr after drug treatment. On the other hand, in animals treated with reserpine only, a blockade of the conditioned avoidance response was observed in 60% of the animals. Almost similar results were obtained in rats receiving the asarone-reserpine combination. In contrast to this, tetrabenazine effectively blocked the action of reserpine.

Effect of α-methyltyrosine treatment on the pharmacological actions of asarone. The solvents employed for dissolving asarone or α-methyltyrosine did not influence the results of any of these experiments.

(*a*) Rectal temperature of mice: treatment of animals with α-methyltyrosine alone did not alter the rectal temperature of mice but, in α-methyltyrosine-pretreated animals, asarone brought about a more intense and prolonged fall in body temperature than observed with asarone alone.

(*b*) Pentobarbitone-induced hypnosis in mice: α-methyltyrosine pretreatment did not cause any change in the pentobarbitone-induced hypnosis in mice (Table 2). Pretreatment of the animals with asarone caused a prolongation in their sleeping time ($P < 0.02$) and this effect was more pronounced in α-methyltyrosine-pretreated animals ($P < 0.001$).

TABLE 2. EFFECT OF ASARONE ON THE PENTOBARBITONE-INDUCED HYPNOSIS OF α-METHYL-L-TYROSINE (AMT) TREATED MICE

Drug	No. of animals	Sleeping time in min ± s.e.
Pentobarbitone sodium	20	62·0 ± 12·0
AMT + pentobarbitone sodium	10	71·0 ± 8·8
Asarone + pentobarbitone sodium	10	132·8 ± 18·6 ($P < 0.02$)*
AMT + asarone + pentobarbitone sodium..	10	212·0 ± 11·0 ($P < 0.001$)†

* P value was calculated by comparing with the group receiving pentobarbitone sodium only.

† P value was calculated by comparing with groups treated with both AMT and pentobarbitone sodium.

(*c*) Conditioned avoidance response of trained rats: Repeated administration of α-methyltyrosine to rats trained for conditioned avoidance response did not influence their acquired behaviour (Table 3). In a dose of 1 mg/kg asarone blocked this response in 30% of the animals, but the

TABLE 3. EFFECT OF ASARONE ON THE CONDITIONED AVOIDANCE RESPONSE (CAR) AND THE ESCAPE RESPONSE (ER) OF TRAINED RATS PRETREATED WITH α-METHYL-L-TYROSINE (AMT)

Drug	Hr after which the animals were examined after treatment with the last dose of: AMT	Hr after which the animals were examined after treatment with the last dose of: Asarone	No. of animals	CAR lost	ER lost
Control (untreated)	—	—	30	0	0
AMT	6	—	20	0	0
Asarone	—	2	10	3	1
AMT + asarone	6	2	10	4	9 ($P < 0.01$)

P value was calculated using χ^2 test by comparing with the results obtained in animals treated with asarone only.

escape response was not significantly influenced. This effect of asarone was markedly enhanced in animals treated with α-methyltyrosine, 90% losing their escape response as well. The effect of asarone on conditioned avoidance response lasted only for 5 hr, but of animals which received α-methyltyrosine and asarone, 40% showed a blocking effect on conditioned avoidance response even when examined after 24 hr.

Discussion

Previous studies in rats have shown that the tranquillizing effect of asarone is not mediated through changes in the brain 5-HT level (Dandiya & Menon, 1963), and unlike reserpine, which produces a stimulant effect in iproniazid-treated animals (Shore & Brodie, 1957), asarone causes sedation in mice treated with iproniazid (Dandiya & Menon, 1964). Since the structure of asarone resembles parts of the reserpine molecule, we wondered whether these two drugs acted on the same receptors to bring about the tranquillizing effect. Quinn, Shore & Brodie (1959) have shown that tetrabenazine, which also acts by lowering catecholamines, if administered before reserpine is capable of blocking its effect, thereby showing that both drugs act on the same receptors. We have found that asarone treatment failed to block the effect of reserpine in rats. More conclusive evidence was obtained in rats trained for the conditioned avoidance response. Whereas pretreatment with tetrabenazine blocked the reserpine effect, in animals which received asarone before reserpine, the blocking effect on the conditioned avoidance response of reserpine was unaffected, proving that the site of action of reserpine and asarone are different. Moreover, unlike reserpine, asarone failed to lower brain noradrenaline.

α-Methyltyrosine, which lowers brain noradrenaline content without affecting the 5-HT level (Spector & others, 1965), enhances some important actions of reserpine and chlorpromazine. It has been suggested that the enhancement is related to the lowered noradrenaline content of the hypothalamus (Menon, Bapna & Dandiya, 1966). The dosage schedule of α-methyltyrosine we have used caused a lowering of brain noradrenaline of rats and mice by 65%. Since noradrenaline has been suggested as a possible neurotransmitter of the ergotropic system of the hypothalamus (Brodie, Spector & Shore, 1959) a reduction in the content of this amine by reserpine and chlorpromazine would allow a further predominance of the trophotropic system in which 5-HT has been suggested as the neurotransmitter. Our present findings show that all three pharmacological actions of asarone were more marked in α-methyltyrosine-treated animals. The explanation for the effect of reserpine and chlorpromazine could be applied also to asarone. The antagonism of asarone to the stimulation produced by the ergotropic system stimulants like lysergic acid diethylamide and amphetamine (Dandiya & Menon, 1964) supports this view. But the ineffectiveness of asarone in lowering either brain 5-HT (Dandiya & Menon, 1963) or noradrenaline suggests that its mechanism of action is different from that of reserpine; it is probably more closely related to

that of chlorpromazine. The fact that both chlorpromazine and asarone effectively antagonize the central excitatory effects of mice treated with iproniazid before reserpine (Dandiya & Menon, unpublished observation) is additional evidence for this possibility.

Acknowledgements. The authors acknowledge with gratitude the financial assistance given by the Indian Council of Medical Research for this project. The authors also wish to acknowledge the generous supply of α-methyl-L-tyrosine by Merck, Sharp and Dohme, Philadelphia, noradrenaline bitartrate by Philips (India) Ltd., Calcutta, reserpine base by CIBA, Basle, and tetrabenazine by Roche Products, Bombay.

References

Baxter, R. M., Dandiya, P. C., Kandel, S. I., Okany, A. & Walker, G. C. (1960). *Nature, Lond.*, **185**, 466–467.
Brodie, B. B., Spector, S. & Shore, P. A. (1959). *Pharmac. Rev.*, **11**, 548–564.
Cook, L. & Weidley, E. F. (1956). *Ann. N.Y. Acad. Sci.*, **66**, 740–752.
Dandiya, P. C. & Cullumbine, H. (1959). *J. Pharmac. exp. Ther.*, **125**, 353–359.
Dandiya, P. C. & Sharma, J. D. (1962). *Ind. J. med. Res.*, **50**, 46–60.
Dandiya, P. C. & Menon, M. K. (1963). *Br. J. Pharmac. Chemother.*, **20**, 436–442.
Dandiya, P. C. & Menon, M. K. (1964). *J. Pharmac. exp. Ther.*, **145**, 42–46.
Dandiya, P. C. & Menon, M. K. (1965). *Life Sci.*, **4**, 1635–1641.
Menon, M. K., Bapna, J. S. & Dandiya, P. C. (1966). *J. Pharmac. exp. Ther.*, in the press.
Nagatsu, T., Levitt, M. & Udenfriend, S. (1964). *J. biol. Chem.*, **239**, 2910–2917.
Quinn, G. P., Shore, P. A. & Brodie, B. B. (1959). *J. Pharmac. exp. Ther.*, **127**, 103–109.
Shore, P. A. & Brodie, B. B. (1957). *Proc. Soc. exp. Biol. Med.*, **94**, 423–435.
Shore, P. A. & Olin, J. S. (1958). *J. Pharmac. exp. Ther.*, **122**, 295–300.
Spector, S., Sjoerdsma, A. & Udenfriend, S. (1965). *Ibid.*, **147**, 86–95.