[Chem. Pharm. Bull. 24(8)1784—1788(1976)]

UDC 547.571.09 : 615.322.076.9 : 581.192

Pharmacological Studies on Chinese Cinnamon. III.[1] Electroencephalographic Studies of Cinnamaldehyde in the Rabbit

Masatoshi Harada, Yuichi Fujii, and Jyoji Kamiya

Faculty of Pharmaceutical Sciences, University of Chiba[2]

(Received October 30, 1975)

Effects of cinnamaldehyde (CA), the main component of Chinese cinnamon which has frequently been prescribed as a remedy in Chinese medicine, on the spontaneous electroencephalograms (EEGs) as well as on the recruiting response and the augmenting response in rabbits were studied. CA converted resting patterns in the EEGs recorded from the frontal cortex, the hippocampus, the amygdala, and the midbrain reticular formation to arousal patterns in the gallamine-paralyzed preparation with the intact brain. In the midpontine pretrigeminal transected preparation, CA also induced an arousal pattern in the electrocorticogram (ECoG). In the low cerveau isolé preparation, CA either converted a resting pattern in the ECoG to a sequence of low voltage fast waves or did not elicit such an action depending upon each individual preparation. In the high cerveau isolé preparation, CA was not capable of producing any effect on the ECoG. CA, in higher doses, inhibited the recruiting response and the augmenting response. It was concluded that CA produced a centrally originating EEG activation through a direct or indirect excitatory action on the brainstem reticular formation.

In the previous paper,[3] it was reported that cinnamaldehyde (CA), the main component of Chinese cinnamon, induced an arousal pattern in the spontaneous electrocorticograms (ECoGs) in unrestrained rabbits with chronically implanted electrodes. CA belongs to a group of the vegetable essential oils and many crude drugs which contain the essential oils as the main components have frequently been used in Chinese medicine. The essential oils are lipophilic and hence it can be thought that they may directly exert some actions on the central nervous system as do centrally acting lipophilic drugs. On the other hand, since many essential oils have local effects on the peripheral organs, it is presumable that they may indirectly influence the central nervous system through their local effects. In the present paper, we studied whether or not the action of CA mentioned above originated in the central nervous system. Moreover, the action of CA on the recruiting response[4] as well as on the augmenting response[5] in the rabbit was studied. Aminopyrine and sodium salicylate were employed as referential drugs because pharmacological effects of CA so far obtained in the previous study[3] somewhat resembled those of these referential drugs and electroencephalographic studies of aminopyrine in the cat have been reported by Ban.[6]

Experimental

Methods——Fifty four rabbits of either sex, ranging from 2.0 to 4.0 kg in weight, were used. After a tracheotomy was performed under ether anesthesia, animals were fixed in a stereotaxic instrument (B-301, Takahashi) and prepared in the following manner. Infiltration of 1% procaine hydrochloride solution was employed at surgical sites during the experimental period whenever necessary. In the case of an arterial cannulation, its procedure was accomplished before the fixation.

1) Part II: M. Harada and S. Yano, *Chem. Pharm. Bull.* (Tokyo), **23**, 941 (1975).
2) Location: *33–1, Yayoi, Chiba, 280, Japan.*
3) M. Harada and Y. Ozaki, *Yakugaku Zasshi*, **92**, 135 (1972).
4) R.S. Morison and E.W. Dempsey, *Amer. J. Physiol.*, **135**, 293 (1942).
5) R.S. Morison and E.W. Dempsey, *Amer. J. Physiol.*, **135**, 281 (1942).
6) T. Ban, *Nippon Yakurigaku Zasshi*, **57**, 448 (1961).

1) **Recording of the Spontaneous Electroencephalograms (EEGs)**——a) The Preparation with Intact Brain: Under artificial respiration after paralysis of the animal with 1 mg/kg of intravenous (*i.v.*) gallamine triethiodide, concentric electrodes measuring 0.8 mm in diameter were placed in the hippocampus (P: 4, L: 4, H: 5.5), the amygdala (A: 3, L: 6, H: −5), and the midbrain reticular formation (P: 9, L: 2, H: −1), according to the stereotaxic atlas for the rabbit brain by Sawyer, *et al.*[7] Two metal screws were employed as cortical electrodes on the frontal cortex.

b) The Midpontine Pretrigeminal Transected (PTG) Preparation: The skull bone just posterior to the supraoccipital ridge was removed to expose a part of the cerebellum. A complete transection along the midpontine-pretrigeminal plane was performed by means of a blunt lancet introduced over the cerebellum. Two metal screws were employed as cortical electrodes on the occipital cortex.

c) The Low Cerveau Isolé Preparation:[8] The occipital bone was removed and a complete transection along the postcollicular-prepontine plane was performed by means of a blunt lancet over the lower edge of the tentorium. Two metal screws were employed as cortical electrodes on the occipital cortex.

d) The High Cerveau Isolé Preparation:[8] The occipital bone was removed and a complete transection along the precollicular-prepontine plane was performed by means of a blunt lancet over the occipital cortex. Two metal screws were employed as cortical electrodes on the frontal cortex. In all preparations (a)—d)) drugs were administered *via* the marginal vein of the ear.

2) **Recording of the Recruiting Response and the Augmenting Response**——The test on the recruiting response and the augmenting response were carried out in the PTG preparation and under an arterial application of drugs. The former response and the latter response were induced by electrical stimulation of the nucleus Centrum medianum (P: 4, L: 4, H: 1) and the nucleus Ventralis posterolateralis (P: 5, L: 2, H: 1), respectively. Both nuclei were stimulated with a square wave pulse of 1 msec duration at a frequency of 8 Hz for 10 sec *via* a concentric electrode through an electronic stimulator (3F-31, Sanei). Voltage ranged from 1.5 to 3 V depending on the sensitivity of each individual preparation. One metal screw served as a recording electrode was placed extradurally in the region of the sensory cortex and another metal screw served as a referential electrode was located in the bone of the frontal sinus. Drugs were administered *via* the internal carotid artery and *via* the vertebral artery. In the case of the internal carotid arterial administration, the method of Trendelenburg[9] was modified. All vessels branching from the commom carotid artery and from the external carotid artery except the internal carotid artery and the lingual artery were ligated and a fine polyethylene tube was proximally cannulated in the lingual artery. The carotid sinus at the operated side was denervated. Drug solutions were administered through this tube into the internal carotid artery under a temporal occlusion of the common carotid artery and the external carotid artery. In the case of the vertebrarterial administration, the method of Kaida[10] was employed. The subclavian artery was exposed and all vessels branching from this artery were ligated leaving the vertebral artery intact. A fine polyethylene tube was inserted proximally in the subclavian artery in order to let drug solutions enter the vertebral artery. In both routes of the drug administration, 0.2 ml of the drug solution followed by 0.2 ml of a flushing saline solution containing 100—150 units of heparin per 1 ml was injected for 30 sec.

In all experiments, EEG recordings were made on a polygraph (141—6, Sanei) and the femoral arterial blood pressure was recorded with a mercury manometer. Drug administration were undertaken after the effect of ether inhaled had been blown off, usually 2 hr after removal of ether. At the end of the experiment the brain was fixed with 10% formalin, sectioned at 20 μ, stained with Luxol fast blue and counterstained with Cresyl violet. The site of electrodes and the transection of the brainstem were confirmed. Also, perfusion of the brain by intra-arterial (*i.a.*) drug solution was confirmed through an application of an ink solution to both arteries.

Drugs CA was emulsified in saline solution of 0.5% carboxymethylcellulose sodium for *i.v.* administration. In the case of i.a. injection, CA was emulsified in saline solution. Solutions of CA were freshly prepared at use. Referential drugs used were as follows: Aminopyrine, eserine sulfate, methamphetamine hydrochloride, and sodium salicylate. Doses below refer to those of the salt except CA and aminopyrine.

Result

I) Effect of CA on the Spontaneous EEGs

a) The Preparation with the Intact Brain——Ten and 20 mg/kg of CA consistently converted resting patterns in the EEGs recorded from the frontal cortex, the hippocampus, the amygdala, and the midbrain reticular formation to arousal patterns within 30 sec after

7) C.H. Sawyer, J.W. Everet, and J.P. Green, *J. Comp. Neurol.*, **101**, 801 (1954).
8) G. Moruzzi, *Electroenceph. clin. Neurophysiol.*, **16**, 2 (1964).
9) U. Trendelenburg, *Brit. J. Pharmacol.*, **9**, 481 (1954).
10) W. Kaida, *Igaku Kenkyu*, **21**, 572 (1951).

the drug administration. These patterns were similar to those induced by an external stimulus like a pinch of the extremities. These arousal patterns returned to the original ones in about 4—6 min after the drug administration. CA, in a dose of 5 mg/kg, did not affect the spotaneous EEGs. Typical tracings are shown in Fig. 1. Ten mg/kg of aminopyrine and 20 mg/kg of sodium salicylate produced arousal patterns similar to those induced by 10 mg/kg of CA in all leads and these arousal patterns lasted for about 4—6 min in both cases. Aminopyrine, in a dose of 20 mg/kg, caused seizure discharges in some cases which persisted in all leads for 30—60 sec.

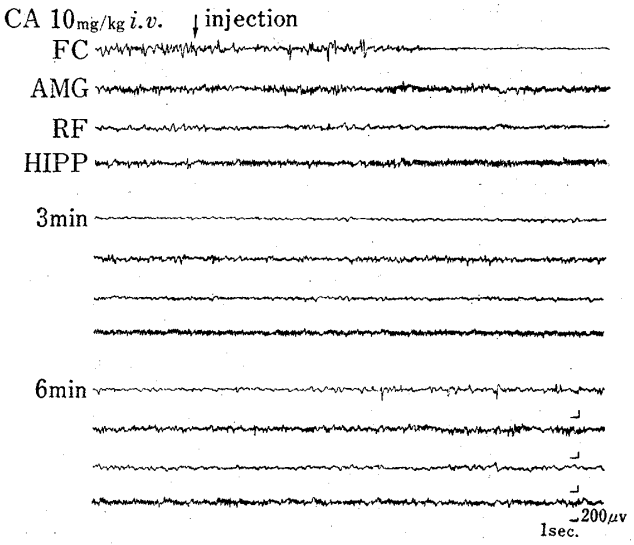

Fig. 1. Effect of Cinnamaldehyde (CA) on the Electroencephalograms in a Gallamine-paralyzed Rabbit

FC: frontal cortex, AMG: amygdala, RF: midbrain reticular formation, HIPP: hippocampus

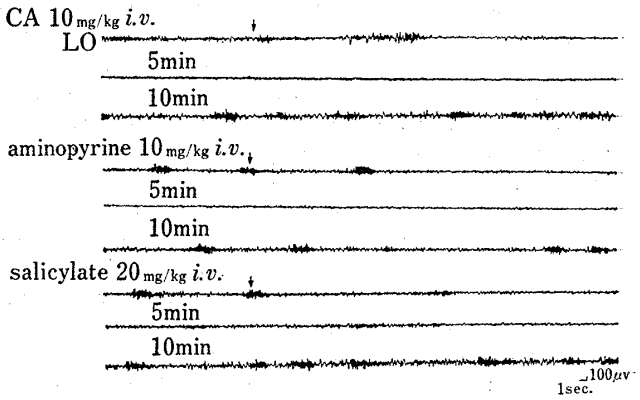

Fig. 2. Effect of Cinnamaldehyde (CA), Aminopyrine, and Salicylate on the Electroencephalogram in the Low Cerveau Isolé Preparation of a Rabbit

LO: left occipital cortex

b) The PTG Preparation——Under the condition in which ECoG tracings consisted of high voltage slow waves, 10 mg/kg of CA converted them to an arousal pattern. This ECoG change recovered to the control pattern in about 6 min after the drug administration. Ten mg/kg of aminopyrine, 20 mg/kg of sodium salicylate, and 0.5 and 2 mg/kg of methamphetamine produced a similar effect.

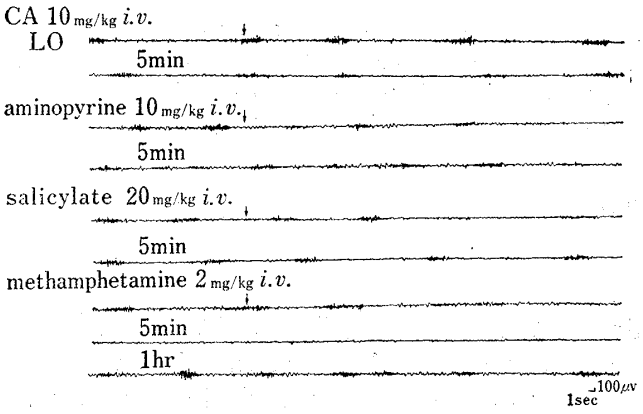

Fig. 3. Effect of Cinnamaldehyde(CA), Aminopyrine, Salicylate, and Methamphetamine on the Electroencephalogram in the low Cerveau Isolé Preparation of a Rabbit

LO: left occipital cortex

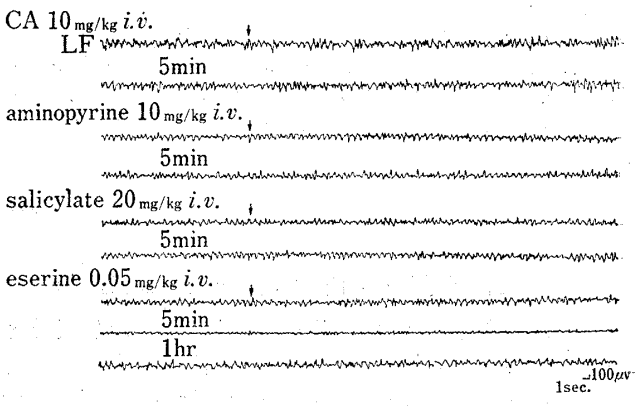

Fig. 4. Effect of Cinnamaldehyde (CA), Aminopyrine, Salicylate, and Eserine on the Electroencephalogram in the High Cerveau Isolé Preparation of a Rabbit

LF: left frontal cortex

c) **The Low Cerveau Isolé Preparation**——ECoG tracings in a resting state in this preparation were composed of low voltage fast waves and spindle lulls which appeared intermittently. CA, in a dose of 10 mg/kg, either converted this resting pattern to a sequence of low voltage fast waves or did not elicit such an action depending on each individual preparation. A high dose of CA as 20 mg/kg, however, induced such an ECoG change in more preparations. Effect of 10 mg/kg of aminopyrine or 20 mg/kg of sodium salicylate was similar to that induced by 20 mg/kg of CA. Methamphetamine, in a dose of 0.5 mg/kg, consistently produced such an ECoG change. Typical illustrations are given in Fig. 2 and Fig. 3.

d) **The High Cerveau Isolé Preparation**——In ECoG tracings of this preparation, high voltage slow waves with intermittently appearing spindle lulls entirely dominated. Any doses of CA, of aminopyrine, and of sodium salicylate up to 20 mg/kg did not affect the ECoG tracings. On the other hand, 2 mg/kg of methamphetamine and 0.05 mg/kg of eserine induced a sequence of low voltage fast waves. Typical tracings are presented in Fig. 4.

2) Effect CA on the Recruiting Response

No significant difference was observed between the effects induced by internal carotid arterial drugs and vertebrarterial drugs. CA showed no activity on the recruiting response in doses of 5.0 and 7.5 mg/head but showed an inhibitory effect on it in a dose of 10 mg/head. This inhibitory effect was long-lasting and the recovery of the response to the original level was not complete even in 60 min. Aminopyrine exerted an inhibitory action on the response in doses of 5.0 and 7.5 mg/head, which disappeared in about 20 min. Typical recordings obtained with vertebrarterial drugs are shown in Fig. 5, in which an example of no inhibitory action by 10 mg/head of CA is illustrated.

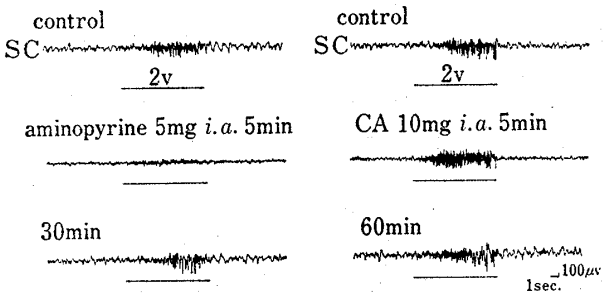

Fig. 5. Effect of Cinnamaldehyde (CA) and Aminopyrine on the Recruiting Response in the Midpontine Pretrigeminal Transected Preparation of a Rabbit

SC: sensory cortex
Drugs were administered *via* the vertebral artery.
The horizontal bar indicates the period of electrical stimulation.

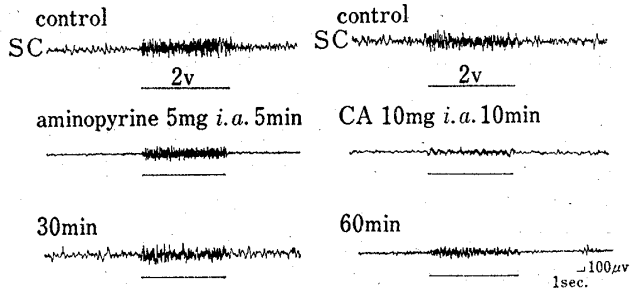

Fig. 6. Effect of Cinnamaldehyde (CA) and Aminopyrine on the Augmenting Response in the Midpontine Pretrigeminal Transected Preparation of a Rabbit

See Fig. 5 for detailed explanations.

3) Effect of CA on the Augmenting Response

The fashion of the effect of CA on the augmenting response was similar to that on the recruiting response in the dose and the duration of action. Aminopyrine exerted no action on the augmenting response even in such higher doses of 7.5 or 10 mg/head as often induce seizure discharges in ECoG. Typical recordings are presented in Fig. 6.

4) Effect of CA on the Blood Pressure

Responses of the blood pressure to 10 and 20 mg/kg, or 5, 7.5, and 10 mg/head, of CA varied individually in all of the preparations, and could be distinguished into three patterns; a fall (5—20 mmHg), an elevation (10—35 mmHg), and an elevation (5—25 mmHg) preceeded by a transient fall. These changes in the blood pressure were not sustained, disappearing within 10 min. In the case of *i.v.* administration of CA, changes in the EEGs and a change

in the blood pressure were observed to occur almost simultaneously. In the case of its *i.a.* injection, however, changes in the EEGs appeared earlier than a change in the blood pressure.

Vehicles exerted no influence on the EEGs and the blood pressure in all experiments.

Discussion

CA, administered through the *i.v.* route, converted resting patterns in the EEGs recorded from the frontal cortex, the hippocampus, the amygdala, and the midbrain reticular formation to similar patterns to those induced by an external sensory stimulus in gallamine-paralyzed rabbits with the intact brain. In the PTG preparation, CA produced a similar arousal pattern in the ECoG. In the low cerveau isolé preparation, however, CA could not elicit such an effect mostly in the minimal dose enough to produce the effect in the above two preparations and higher doses were needed in order to make its appearance more frequent. Moreover, in the high cerveau isolé preparation, CA could not produce the effect entirely. Through all experiments carried out under the *i.v.* administration of CA, no relationship between changes in the blood pressure and the appearance of EEG arousal patterns was recognized. Furthermore, in the case of its *i.a.* administration in the PTG preparation, whether via the internal carotid artery or via the vertebral artery, an arousal response in the ECoG was observed to take place earlier than a change in the blood pressure. These findings indicate that CA produced an EEG activation through an excitatory action on the brainstem reticular formation directly or indirectly, *e.g.* through the hypothalamic activating system.[11] Aminopyrine and sodium salicylate caused the same effects as those induced by CA in the EEGs. The potency of aminopyrine, CA, and sodium salicylate to produce such effects decreased in that order on the weight basis. Ban[6] demonstrated that aminopyrine produced an EEG activation in the cat with the intact brain and that it lost this effect in the cerveau isolé preparation, discussing an important role of the brainstem reticular formation on this action.

In the PTG preparation and under both kinds of the *i.a.* administration, CA, in higher doses, inhibited the recruiting response and the augmenting response. On the contrary, aminopyrine markedly inhibited the former response but did not affect the latter response. Ban[6] also reported that aminopyrine inhibited the recruiting response and exerted a weak inhibition on the augmenting response in the cat, and concluded that these inhibitory actions would be due to an action of this drug on the reticular formation. Our results indicated a difference of the action between CA and aminopyrine on these evoked responses. It seems that a non-specific action of CA (*e.g.* a local anesthetic action (unpublished observation)) might play a part in such inhibitory actions.

Acknowledgement We gratefully acknowledge the support of our research by a Grant-in-Aid from Hoansha. Also thanks are due to Dr. Y. Ishii, the Research Laboratories, Nippon Kayaku Co., for his kind help.

11) H. Kawamura, Y. Nakamura, and T. Tokizane, *Japan. J. Physiol.*, **11**, 564 (1961).