THE CHOLINOLYTIC ACTION OF CAMPHOR

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One of the important properties of camphor responsible for its cardiotonic action is its cholinolytic activity. In hypoxia associated with cardiac failure the concentration of acetylcholine in the heart muscle is considerably increased. Acetylcholine lowers the oxygen consumption of the heart [9], as a result of which its energy production is still further reduced. The tonic effect of camphor on the heart when the myocardium is in a hypoxic state is evidently due to an increase in energy formation in the heart and to a change in neuro-humoral relationships towards depression of acetylcholine activity.

In face of these considerations, an investigation was made of the peripheral and central cholinolytic action of levorotatory camphor.

EXPERIMENTAL METHOD

The action of camphor on the peripheral sympathetic ganglia was studied in experiments in which changes in the blood pressure and in the tone of the nictitating membrane were recorded during stimulation of the cervical sympathetic nerve of the cat before and after the administration of camphor. The reactions of the adrenergic and the muscarine-like (M) cholinergic systems were tested by means of adrenaline and acetylcholine. The changes in the circulation, respiration, and the nictitating membrane in response to the intravenous injection of cytisine were used as an indicator of the effect of camphor on the peripheral nicotine-like (N) cholinergic systems.

Arecoline and nicotine convulsions in albino mice, associated with the central action of these drugs, were used as models for testing the central cholinolytic effect of camphor. The convulsive movements of the animals were recorded on an actograph. Arecoline (25 mg/kg) and nicotine (20 mg/kg) were injected intraperitoneally. Altogether 232 experiments were performed.

For the comparative evaluation of the central and peripheral cholinolytic action of camphor the method described by P. P. Denisenko [1] was used; experiments were conducted on unanesthetized cats immobilized with the curare-like drug ditilin (0.3-0.5 mg/kg). At the same time recordings were made of the EEG (in the right and left sensorimotor and the right optic areas) and the ECG (lead II) on a type 4 EEG-1m electroencephalograph. The vagus nerve, placed in a special holder on platinum electrodes, was stimulated by rectangular pulses with a frequency of 50 per sec, a duration of 10 msec, and a voltage of 0.75-3 V for a period of 3-5 sec. The EEG and ECG were recorded before and for 60-90 min after the subcutaneous injection of camphor (0.1-0.3 g/kg) in the form of an oily solution. The vagus nerve was stimulated every 5 min so that the beginning and end of the cholinolytic effect of camphor could be studied.

The sulfhydryl groups in the homogenate and in the choline-reactive protein of the isolated frog's heart and in the superior cervical sympathetic ganglion of the cat were determined by the method of mercurimetric titration [4, 8]. The choline-reactive protein was separated by precipitation with mercuric chloride and the functional groups were then reduced with unithiol [5].

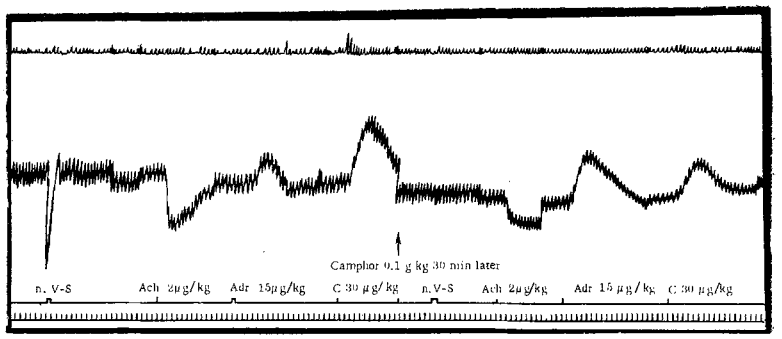

Fig. 1. Effect of camphor on adrenergic and cholinergic systems (in a cat weighing 2.4 kg). From top to bottom: respiration, blood pressure, zero line, time marker (5 sec). n.V-S—electrical stimulation of the vagosympathetic nerve; Ach—injection of acetylcholine; Adr—injection of adrenalin; C—injection of cytisine.

EXPERIMENTAL RESULTS

Comparison of the peripheral M and N cholinolytic activity of camphor (determinations made in 18 cats) showed that 20-60 min after the subcutaneous injection of camphor in a dose of 0.1 g/kg the reaction of the nictitating membrane of the cat to stimulation of the sympathetic nerve supplying it was depressed by 31.7%; the depressor reaction to electrical stimulation of the peripheral segment of the cervical trunk of the vagus nerve was lowered by 21%, and the reaction to injection of acetylcholine by 24.3%. The contraction of the nictitating membrane and the increase in blood pressure observed under the influence of cytisine were depressed on the average by 21% under the influence of camphor (Fig. 1). Consequently, camphor caused approximately equal blocking of the peripheral M and N cholinergic systems, and its ganglion-blocking action was slightly stronger on the sympathetic ganglia.

Administration of camphor in a dose of 5 mg/100 g did not prevent the convulsant action of arecoline, demonstrating that camphor has no central M cholinolytic activity. The preliminary administration of camphor almost completely prevented the development of nicotine convulsions. Only in individual experiments did nicotine produce weak convulsive movements 2-5 min after its administration. All the animals survived. Camphor thus possesses peripheral cholinolytic activity and also has a central N cholinolytic action.

For a comparative assessment of the cholinolytic properties of camphor the method described elsewhere [1] was used, in which the strength and duration of the central and peripheral cholinolytic activity of the tested substances can be determined simultaneously. During stimulation of the vagus nerve a clear arousal reaction appears on the EEG and the heart rate is slowed.

Subcutaneous injection of camphor (0.1-0.3 g/kg) caused no changes in the cortical potentials and did not affect the work of the heart. The "sleep volleys" in the EEG, characteristic of the central cholinolytics, and the slowing of the rhythm of the cortical potentials with an increase in their amplitude were not observed in any experiment.

Stimulation of the vagus nerve against the background of the preliminary injection of camphor in most animals [11 of 14] caused a clear activation reaction in the cortical leads. Only in three experiments was a weak reaction observed, especially in the optic area. The peripheral cholinolytic action of camphor was as a rule quite distinct: the heart rate hardly changed in response to stimulation of the vagus nerve (Fig. 2).

It may be supposed that in doses having a peripheral cholinolytic action camphor does not block the central cholinergic systems of the cat. However, there is another, more probable explanation of the absence of any manifestation of the central N cholinolytic action of camphor on the EEG. We know that substances blocking only the central N cholinergic systems do not, even in large doses, prevent the activation reaction produced by anticholinesterase drugs and nicotine, whereas they do abolish nicotine convulsions. Evidently, the M cholinergic systems of the reticular formation of the brain play a part in the mechanism of the activation reaction produced by nicotine [2]. It may be supposed that the activation reaction of the cerebral cortex resulting from stimulation of the vagus nerve is not associated with the N cholinergic systems of the brain, on which camphor exerts its cholinolytic action. That is why camphor, while blocking nicotine convulsions. does not influence the activation reaction arising in response

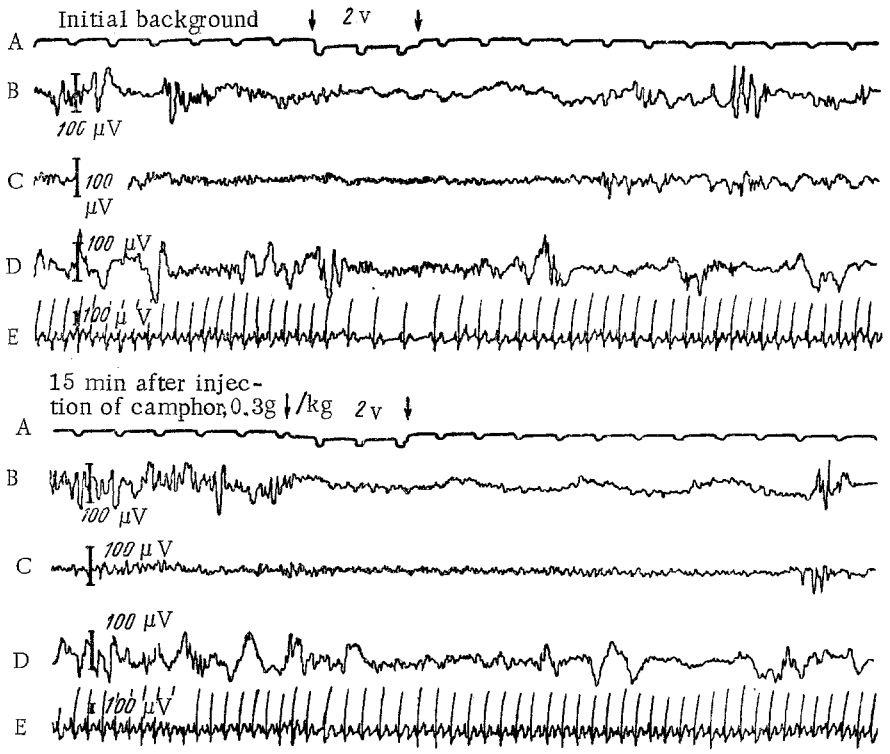

Fig. 2. Effect of camphor on the EEG and ECG during stimulation of the vagus nerve. A) Time marker (1 sec); B) right optic area; C) left sensorimotor area; D) right sensorimotor area; E) ECG (lead II); ↓↑) beginning and end of stimulation of the vagus nerve.

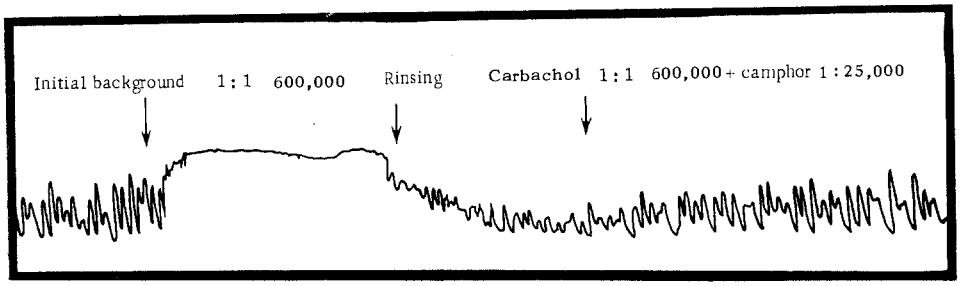

Fig. 3. Cholinolytic action of camphor on the isolated segment of small intestine of the rabbit.

to stimulation of the vagus nerve. To analyze the biochemical nature of the cholinolytic effect of camphor certain enzyme poisons were used in experiments on the isolated small intestine of the rabbit [3]: monoiodoacetic acid (1:10,000), sodium fluoride (1:40,000), and 2,4-dinitrophenol (1:8000).

Camphor, in a concentration of 1:25,000, blocked the spastic contraction of the intestine arising during perfusion with carbachol solution (Fig. 3). Monoiodoacetic acid and sodium fluoride did not influence the cholinomimetic effect of carbachol, but inhibited the cholinolytic action of camphor. This reaction was stronger in experiments when sodium fluoride was used for perfusion and much weaker in experiments with monoiodoacetic acid. These results may be interpreted if it is remembered that monoiodoacetic acid paralyzes trios-phosphate dehydrogenase and arrests the formation of phosphoglyceric acids. Sodium fluoride, on the other hand, stimulates the formation of phosphopyruvic acid from phosphoglyceric acid. Evidently the process of formation of phosphoglyceric acids is of definite importance in the mechanism of the cholinolytic action of camphor.

The uncoupling of oxidative phosphorylation by means of 2,4-dinitrophenol prevents the cholinomimetic effect of carbachol and the cholinolytic action of camphor. Admittedly, against the background of the action of 2,4-dinitrophenol, carbachol caused a spastic contraction of the segment of intestine, but this effect was transient. Evidently by disturbing respiratory phosphorylation, 2,4-dinitrophenol deprives the muscle of material providing the energy required for performing mechanical work.

Metabolic poisons inhibiting oxidative phosphorylation are known to cause ATP breakdown and to lower its content in the smooth muscle. As a result, the muscle becomes incapable of reacting to substances causing it to relax, although the reaction to substances producing contraction persists. Evidently the cholinolytic action of camphor and the cholinomimetic effect of carbachol require different levels of high-energy phosphates in the smooth muscle.

Sulfhydryl groups are of considerable importance in the realization of the specific activity of the enzymes concerned in the mechanism of the reaction of acetylcholine with the cholinergic systems. The interaction between acetylcholine and the cholinergic receptor is accompanied by a decrease in the reactivity of the sulfhydryl groups of the cholinoreceptor protein [5, 6], indicating a change in the structure of the molecule of the receptor protein under the influence of acetylcholine. In conditions in which the sulfhydryl groups are blocked by thiol poisons, the heart muscle does not react to acetylcholine or to stimulation of the vagus nerve.

As a cholinolytic substance, camphor evidently must interact with the cholinergic receptor, preventing it from reacting with acetylcholine and with other substances acting in a similar way to acetylcholine. To test this hypothesis experiments were carried out to determine the concentration of sulfhydryl groups in a homogenate and in the cholinoreactive protein of the isolated frog's heart and also in the superior cervical sympathetic ganglion of the cat.

Camphor in a concentration of 1:5000, like acetylcholine, lowered the concentration of sulfhydryl groups in the homogenate of the isolated frog's heart on the average by 15% ($P < 0.02$). In experiments in which acetylcholine acted against the background of camphor, no significant change took place in the concentration of sulfhydryl groups ($P = 0.12$). A similar picture was observed also in the experiments with cholinoreactive protein.

The existence of antagonism between camphor and acetylcholine shows that these compounds evidently act on the same part of the cholinergic receptor molecule—the active center. The blocking by camphor of the action of acetylcholine must be attributed to the fact that the reaction between camphor and the receptor is accompanied by a change in the structure of the receptor, preventing the interaction between the cholinergic receptor and the mediator. Comparison between the change in the functional state of the cells of the superior cervical sympathetic ganglion of the cat and the concentration of sulfhydryl groups in the ganglion showed that camphor (0.03 g/kg), besides blocking the transmission of nervous impulses in the superior cervical sympathetic ganglion, also depressed the level of sulfhydryl groups in the ganglion by 29.6%.

Evidently camphor, when it acts on the N cholinergic systems of the ganglion, inhibits the activity of the thio compounds of the cholinergic receptor, adversely affecting the transmission of excitation between the neurons.

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