COMBINATION EFFECT OF COMPETITIVE GANGLION BLOCKING AGENTS ON THE NICOTINE-INDUCED GANGLION BLOCKADE IN THE CAT SUPERIOR CERVICAL GANGLION

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Concerning the action of nicotine on the superior cervical ganglion of cat, the assumption of Paton and Perry (1) has been accepted by many workers. According to them nicotine has a dual action, that is, initial depolarizing block followed by a late phase of competitive blockade similar to that of hexamethonium. On the other hand Pellikan (2) suggested that the ganglionic blockade by nicotine is due to the inhibition of acetylcholine release from the terminals of the preganglionic cervical sympathetic nerve fibers.

Assuming that the ganglionic blocking mechanism of nicotine during the late phase block is the same as that of the competitive blocking agents, e.g. hexamethonium, the potency of ganglionic blockade by the combined injection of nicotine and a competitive blocking agent to the superior cervical ganglion must be equal to or less than the addition of each ganglionic blocking potency of the two compounds.

The present paper describes the experiments which were made quantitatively for ganglionic blockade in order to ascertain whether the late phase block by nicotine is due to the same mechanism of action as that of the competitive blocking agents.

MATERIALS AND METHODS

Hexamethonium bitartrate (C_6), tetraethylammonium bromide (TEA), thiamine hydrochloride (thiamine), *l*-nicotine (nicotine), acetylcholine chloride (ACh), pilocarpine hydrochloride (pilocarpine), arecoline hydrobromide (arecoline) and atropine sulfate (atropine) were used immediately after being dissolved in saline.

Cats, weighing 2-4 kg, were anesthetized with 30 mg/kg of intraperitoneal pentobarbital sodium. Contractions of the nictitating membrane were recorded with a front writing-point lever on kymograph.

Preganglionic fiber of ipsilateral cervical sympathetic nerve was dissected free from the vagal nerve and the peripheral cut end was stimulated electrically with the bipolar electrode. Supramaximum stimulation of 20 per second, duration of 1 millisecond, for 6 seconds was made on the preganglionic nerve every 5 minutes.

Intraarterial injection was made in a certain volume of 0.2 ml through the cervical end of the lingual artery into the blood supply of the superior cervical ganglion following the method of Trendelenburg (3). The extracarotid artery was clamped and supra-maximum stimulation of the preganglionic nerve was given one minute after the intra-arterial injection. The clamp was released 20 seconds after the preganglionic stimulation.

Intravenous administration was made through a femoral vein at a constant speed for 10 seconds immediately after the recovery of the contraction response of the nictitating membrane due to the preganglionic stimulation.

For the evaluation experiments with ganglionic blockade intravenous doses of 4 mg/kg of TEA, 1.5 mg/kg of C_6 and 30 mg/kg of thiamine were used as an effective dose to depress the contraction response of the nictitating membrane to the preganglionic supramaximum stimulation and to the intraarterial injection of 100 μ g of ACh as reported previously (4).

The potency of ganglionic blockade was calculated by the modified method of McIsaac and Millerschoen (5). The decrease of contraction height of the nictitating membrane was expressed as a per cent of control. Each value of per cent block was summed up

together until the complete recovery of the contraction was observed. This summed up value was expressed as total blocking value (TBV).

RESULTS

1) Combination effect of TEA and C_6

After observing the effect of single dose of 4 mg/kg of TEA and 1.5 mg/kg of C_6 on the contraction response of the nictitating membrane elicited by preganglionic electric stimulation, the TBV of combination of both drugs was measured by the simultaneous administration. The TBV of the combination of the two compounds was less than the addition of each TBV of TEA and C_6 (Fig. 1).

2) Effect of TEA on the action of nicotine

Intraarterial 150 μ g of nicotine and intravenous 4 mg/kg of TEA were injected. The TBV of the combined dose of TEA and nicotine was larger than the addition of each TBV of nicotine and TEA (Fig. 2). By the preadministration of TEA

FIG. 1. Combination effect of TEA and C_6 on the contraction response of cat nictitating membrane. Preganglionic supramaximum stimulations were given every 5 minutes. TEA: 4 mg/kg, i.v. C_6 : 1.5 mg/kg, i.v. TBV: total blocking value in %·5 minutes.

FIG. 2. Effect of TEA on the ganglionic action of nicotine. Condition of the stimulations was as under Fig. 1. N: 150 μ g of nicotine into the blood supply of superior cervical ganglion. TEA: 4 mg/kg, i.v. TBV: as under Fig. 1.

FIG. 3. Effect of C_6 on the ganglionic action of nicotine. Condition of the stimulations was as under Fig. 1. N: 150 μ g of nicotine into the blood supply of superior cervical ganglion. C_6 : 1.5 mg/kg, i.v. TBV: as under Fig. 1.

the ganglion-stimulating effect of intraarterial nicotine was abolished.

3) Effect of C_6 on the action of nicotine

The results obtained in this experiments were similar to those obtained by TEA and nicotine. The TBV of the combination of 150 μ g of intraarterial nicotine and 1.5 mg/kg of C_6 was larger than the addition of each TBV of nicotine and C_6 (Fig. 3).

4) Blocking effect of nicotine on the contraction to the intraarterial ACh

As shown in Fig. 4 the alternate contraction responses of the nictitating membrane to preganglionic supramaximum electric stimulation and to intraarterial 100 μ g of ACh were completely blocked immediately after the intraarterial injection of 150 μ g of nicotine, and these blocks recovered to normal with the same pace.

5) Effect of nicotine in combination with C_6 on the ganglion stimulating action of the muscarinic substances

After recording the control responses to the preganglionic supramaximum stimulation, intraarterial injection of 40 μ g of pilocarpine was administered, resulting in the

FIG. 4. Blocking effect of nicotine on the contraction response of the nictitating membrane to intraarterial ACh. The marks of point and small arrow show preganglionic electric stimulation and intraarterial 100 μ g of ACh, respectively. N: 150 μ g of nicotine into the blood supply of superior cervical ganglion.

FIG. 5. Effect of nicotine in combination with C_6 on the ganglion-stimulating action of pilocarpine. The point marks are as in Fig. 4. Forty μ g of pilocarpine (P), 1 mg of C_6 and 150 μ g of nicotine (N) were injected into the blood supply of superior cervical ganglion.

contraction of the nictitating membrane of which duration was more prolonged than that of the response to preganglionic stimulation (Fig. 5). Intraarterial injection of 1 mg of C_6 did not abolish the contraction response to pilocarpine, but inhibited the response to preganglionic stimulation. This contraction response to pilocarpine was not affected by 150 μ g of intraarterial nicotine after the administration of 1 mg of C_6 (Fig. 5).

Similar results were obtained with arecoline. A prolonged contraction response to intraarterial 200 μ g of arecoline which was not antagonized by the preadministration of C_6 was not affected by intraarterial nicotine in combination with C_6 (Fig. 6). These prolonged ganglion-stimulating actions of pilocarpine and arecoline were completely abolished by the preadministration of atropine.

FIG. 6. Effect of nicotine in combination with C_6 on the ganglion-stimulating action of arecoline. The point marks are as in Fig. 4. Two hundreds μg of arecoline was injected into the blood supply of superior cervical ganglion. The doses of C_6 and nicotine (N) are the same as under Fig. 5.

6) Effect of thiamine on the action of nicotine

Ganglion-stimulating effect of intraarterial 150 μg of nicotine was hardly depressed by the preadministration of 30 mg/kg of thiamine. The TBV of the combination of nicotine and thiamine was almost the same as the addition of each TBV of these two compounds (Fig. 7).

DISCUSSION

As a proof experiment, about 50 per cent blocking doses of TEA or C_6 were used intravenously referring the previous report (4). When C_6 was combined with TEA, the TBV of the combination was less than the addition of the TBVs of TEA and C_6 . In the previous report (4) it has been shown that TEA and C_6 in such doses did not show any effect on the contraction response of the nictitating membrane to postganglionic stimulation. It has been also shown that these agents could block the contraction response of the nictitating membrane to the intraarterial 100 μg of ACh in the same degree as in the case of the electric stimulation. Therefore the

FIG. 7. Effect of thiamine on the ganglionic action of nicotine. Condition of the stimulations was as under Fig. 1. N: 150 μg of nicotine into the blood supply of superior cervical ganglion. T: 30 mg/kg of thiamine, i.v. TBV: as under Fig. 1.

combination blocking-effect of TEA and C₆ must occur from the ganglionic origin. Furthermore, from the results obtained from the combination of TEA and C₆, it appears that these two compounds acted in the similar mechanism and that the site of action of TEA and C₆ in the doses employed in the present study was defined on the similar receptor sites to nicotine of the ganglion cell.

On the other hand when nicotine was injected after the administration of TEA, the TBV of the combination of TEA and nicotine was larger than the addition of each TBV of nicotine and TEA. The similar result was obtained with the combination of nicotine and C₆. The contraction response to intraarterial 100 μ g of ACh was blocked and this response recovered with the same pace to the recovery of the response to pre-ganglionic electric stimulation. This result indicates that nicotine acted on the ganglion cell as a competitive blocking agent in the late phase block. However, it has been shown that the initial stimulating action of nicotine was abolished by the combination with TEA or C₆. These results may indicate that the receptors were occupied by C₆ or TEA and then nicotine could not combine with the receptors of the ganglion cells. The late phase blockade by nicotine may occur on a site of action different from that of TEA and C₆ at the ganglion.

Jones (6) suggested that the attachment of endogenous ACh to muscarinic receptors at the ganglion cell may serve to modify the normal ganglionic transmission initiated by an action of ACh on the nicotinic receptors. In this respect the effect of nicotine on the muscarinic receptor has also been investigated. After a larger dose of C₆ was administered intraarterially, nicotine showed no initial stimulation and could not affect the prolonged ganglion stimulating action of pilocarpine or that of arecoline. Thus it is likely that nicotine acted on another sites different from the muscarinic and nicotinic receptor sites of the ganglion cell, when nicotine was used by combined injection with TEA or C₆.

Thiamine which has been reported to be an antagonist of nicotine and have a competitive ganglionic blocking property (4) was also administered intravenously in combination with nicotine, and then it was found that this combination effect was additive. Thiamine hardly blocked the initial stimulation of nicotine. It is evident that nicotine acted on the nicotinic receptor by depolarization. In contrast with the combination effect of nicotine and TEA or nicotine and C₆, the TBV of the combination of nicotine and thiamine was equal to the addition of each TBV of nicotine and thiamine. If the potentiation by the combination of nicotine and TEA or nicotine and C₆ was attributable to the inhibitory effect of nicotine to the blood supply of the ganglion (2), the value of the combination of nicotine and thiamine also has to be larger than that obtained in the present study. This question is excluded by the fact reported here that the duration of the combination effect of nicotine with one of the competitive blocking agents was longer than that of the action of nicotine alone.

By the evaluation of the TBV, it is obvious that the ganglion blocking effect of nicotine was potentiated strongly in combination with TEA or C₆. Therefore, if the

nicotine blockade was not resulted from a competitive mechanism on the receptor of the ganglion cell, this potentiation may due to the action of nicotine on the preganglionic nerve terminals. It appears that the molecules of nicotine which could not combine with the receptor of the ganglion cell were acted on the preganglionic nerve terminals to inhibit the release of ACh. This assumption has a discrepancy on the data obtained with the combination of thiamine with nicotine, but thiamine may have an antagonistic action to the inhibitory effect of nicotine of the release of ACh from the preganglionic nerve terminals. The results obtained in the present investigation support an assumption that the nicotine blockade at the late phase is due to an inhibition of the ganglionic transmission both by a mechanism of the competition on the receptor site of the ganglion and by the depression of the ACh release from the preganglionic nerve terminals.

SUMMARY

An attempt was made to know whether the late phase block of nicotine on the superior cervical ganglion is due to the same mechanism of action as that of the competitive blocking agents by a quantitative comparing method.

From the evaluation of the total blocking value, it was found that the combination effect of nicotine with TEA or C₆ was strongly potentiated while the combination effect of TEA and C₆ was less than the addition of their values. It has been shown that nicotine did not affect the contraction responses to muscarinic substances injected to the superior cervical ganglion. These facts may indicate that the sites of action of nicotine at the late phase block are both the receptor site of the ganglion cell and the preganglionic terminals.

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