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IDENTIFICATION OF THE SMOOTH MUSCLE EXCITATORY RECEPTORS FOR ERGOT ALKALOIDS

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In cats under sodium pentobarbitone anaesthesia the first dose of ergotamine (50 μg/kg) invariably caused retraction of the nictitating membrane and a rise of arterial blood pressure. However, the responses to the dose of ergotamine were strikingly reduced when the cats were previously treated with the adrenaline antagonists phenoxybenzamine (5 mg/kg) or ergotamine (50 μg/kg). Further experiments to identify receptors for ergotamine were carried out on three different isolated smooth muscle preparations: rabbit aorta, rat uterus and dog retractor penis. Receptors for adrenaline were selectively protected by high concentrations of adrenaline throughout exposure of the preparation to a blocking concentration of ergotamine or phenoxybenzamine. Protected muscles responded to ergotamine ; unprotected muscles did not. Muscles where receptors for acetylcholine, histamine or 5-hydroxytryptamine were protected by high concentrations of these drugs did not respond to ergotamine. Ergometrine, which has no blocking action on adrenaline receptors, behaved in the same way as ergotamine ; muscles which were protected by adrenaline against blockade by phenoxybenzamine responded to ergometrine, but unprotected muscles did not. The stimulant actions of adrenaline, ergotamine and ergometrine were also protected against the blocking action of phenoxybenzamine by treating the muscle with a high concentration of ergometrine instead of adrenaline. It is concluded that, in smooth muscle which can be excited by adrenaline, ergotamine and ergometrine act by combining with adrenaline receptors, and that ergotamine may therefore be regarded not only as an adrenaline antagonist but also as a partial agonist since it excites the same receptors.

Ergot alkaloids directly stimulate smooth muscle in many organs, generally causing sympathomimetic effects, such as retraction of the nictitating membrane and increase of blood pressure (Dale, 1906). Smooth muscle is known to have specific and quite discrete receptors for at least four types of agonist, acetylcholine, histamine, 5-hydroxytryptamine and sympathomimetic amines (Furchgott, 1954), but there is no direct evidence to identify receptors for the ergot alkaloids.

Some of the ergot alkaloids are also competitive antagonists of sympathomimetic amines which excite smooth muscle (Dale, 1906). These alkaloids must therefore react with the adrenaline α receptors to exert their blocking action. It is possible that this interaction with adrenaline receptors may result in stimulation of the smooth muscle as well as in blockade of sympathomimetic amine action. This hypothesis agrees with observations that ergot alkaloids with an adrenergic blocking action can

antagonize their own stimulant action and that of other ergot alkaloids. Barry (1937) and Linegar (1940) reported that the pressor response to an initial dose of ergotamine was not repeated when subsequent doses were given. Rothlin (1946) showed that dihydroergotamine antagonized the stimulant action of ergobasin and ergotamine.

In the present paper evidence is presented to show that the ergot alkaloids ergotamine and ergometrine stimulate smooth muscle by interaction with the adrenaline α receptors.

METHODS

Blood pressure and nictitating membrane of the cat. Cats of either sex were anaesthetized with sodium pentobarbitone (45 mg/kg intraperitoneally). Artificial respiration was applied by a Palmer Ideal pump through a tracheal cannula. Kymographic records of femoral arterial blood pressure were made with a mercury manometer. Nictitating membrane contractions were recorded with isotonic gimbal levers at a tension of 5 g and an amplification of 25 times. Injections of ergotamine and phenoxybenzamine (Dibenzyline) were made into the femoral vein.

Receptor protection in isolated organs. The selective receptor protection technique of Furchgott (1954) was used to distinguish receptors for ergotamine and ergometrine. This technique can distinguish between the acetylcholine, histamine, 5-hydroxytryptamine and adrenaline receptors of smooth muscle in strips of rabbit aorta. An isolated smooth muscle preparation is exposed to a concentration of Dibenamine which can block the actions of all four agonists. A high concentration of one of the agonists is placed in the organ bath before addition of the blocking drug and remains in the bath until the blocking drug is washed out. After the bath is washed out, the agonist which has been used for pretreatment can stimulate the muscle whereas agonists of other kinds cannot. It is presumed that the selected agonist in high concentrations occupies a large proportion of the receptors for this agonist, thus preventing access of the blocking agent to these receptor sites but not to the receptor sites for other agonists (Nickerson, 1959).

The blocking agents used were ergotamine and phenoxybenzamine. During exposure of the muscle to these drugs the receptors were protected by either adrenaline or ergometrine. The responses of the preparation to adrenaline, ergotamine and sometimes ergometrine were tested after removal of the blocking agent. Receptors for acetylcholine, histamine and 5-hydroxytryptamine were protected in the same way to test for possible interaction of these receptors with the ergot alkaloids.

Smooth muscle preparations of three types were used, rabbit aorta, dog retractor penis and rabbit uterus. Spiral strips from young female rabbits were prepared as described by Sherrington (1919) and by Furchgott & Bhadrakom (1953). The dog retractor penis muscle consists of smooth muscle in the anterior three-fifths and of a mixture of smooth and skeletal muscle in the posterior two-fifths (Fisher, 1917). Strips of smooth muscle were prepared only from the anterior half, usually from puppies of 1.5 to 4 kg; each animal easily provided two strips. The rabbit uterus preparation consisted of segments of about 2.5 cm length.

Aortic strip and retractor penis preparations were suspended in Krebs-Henseleit solution. Aortic strip preparations were kept at 38° C. For the retractor penis preparation the temperature was reduced to 28° C to minimize the spontaneous contractions which were regularly present at higher temperatures. At 28° C the muscle also became unresponsive to acetylcholine, although responses to adrenaline, histamine and 5-hydroxytryptamine were little, if at all, affected. Preparations of rabbit uterus were suspended in modified Dale solution at 28° C. The solution contained 9 g sodium chloride, 0.42 g potassium chloride, 0.06 g calcium chloride hexahydrate, 0.5 g sodium bicarbonate and 0.5 g glucose/l. This solution abolished spontaneous movements, but had little effect on the responses to drugs. The

solutions for all three preparations were aerated by 95% oxygen with 5% carbon dioxide. In all preparations isotonic contractions at a tension of 1 g were recorded with 5.5 times amplification.

For every experiment two preparations from the same organ were suspended, each in an individual organ bath. Both preparations were exposed to the same concentration of blocking agent, but one was protected throughout the entire period of exposure (5 min) by a high concentration of a selected agonist which was added to the organ bath 5 min before the blocking agent. The second preparation, which was not protected from blockade, served as control.

Drugs. Quantities or concentrations of l-adrenaline bitartrate, acetylcholine chloride, 5-hydroxytryptamine creatinine sulphate and histamine diphosphate refer to the free bases. Quantities or concentrations of ergotamine tartrate, ergometrine maleate and phenoxybenzamine hydrochloride refer to the salts. A 2.5% solution of phenoxybenzamine hydrochloride (Dibenzyline) in propylene glycol was kept as a stock solution and suitable dilutions in 0.9% sodium chloride solution were made freshly on the morning of use.

RESULTS

Effects of ergotamine on blood pressure and nictitating membrane of the cat. Intravenous injection of a first dose of ergotamine (50 μg/kg) in the cat invariably caused a rise in arterial blood pressure and a contraction of the nictitating membrane. The rise in blood pressure varied from 40 to 120 mm Hg, but was generally transient, lasting only 10 to 15 min. The contraction of the nictitating membrane varied widely between cats (3 to 70 mm) and, in contrast to the pressor response, was generally prolonged. In experiments where subsequent doses of ergotamine or phenoxybenzamine were not given the increase in tone was maintained over 2 to 3 hr.

When a second injection of 50 μg ergotamine/kg was given 15 to 120 min after the first injection, an increase in blood pressure comparable to the previous response was never seen, although the level of blood pressure was usually the same at the time of each injection. In each of 8 experiments the blood pressure showed only a relatively small increase. In most cases the pressor response was no more than 10 to 25 mm Hg, but in one experiment where the first dose had given the exceptionally large increase of 120 mm Hg, the second dose caused a rise of 45 mm Hg. The nictitating membrane, still showing an increase in tone due to the first dose, seldom contracted further in response to the second dose, although the maximal contraction shown earlier in the experiment in response to adrenaline had not been reached. The effect of subsequent injections of ergotamine on the arterial blood pressure or nictitating membrane was never greater than that produced by the second dose.

Effects of phenoxybenzamine on the responses to ergotamine in the cat. Because the effects of repeated equal doses of ergotamine were never comparable in magnitude, it was not possible to compare the effects of equal doses of ergotamine before and after blockade of adrenaline receptors with phenoxybenzamine. Phenoxybenzamine (5 mg/kg) was therefore given 20 min before the first dose of ergotamine in 6 experiments. It was first ensured in each experiment that adrenaline in doses of 2 to 20 μg induced pressor responses and contractions of the nictitating membrane. Adrenaline (20 μg) failed to elicit these responses 5 min after injection of phenoxy-

benzamine. In these experiments ergotamine (50 μg/kg) had no effect on the arterial blood pressure in 3 cats ; in the remaining 3 cats the increase was no more than 10 to 15 mm Hg. In all 6 cats the dose of ergotamine failed to cause any contraction of the nictitating membrane (Fig. 1).

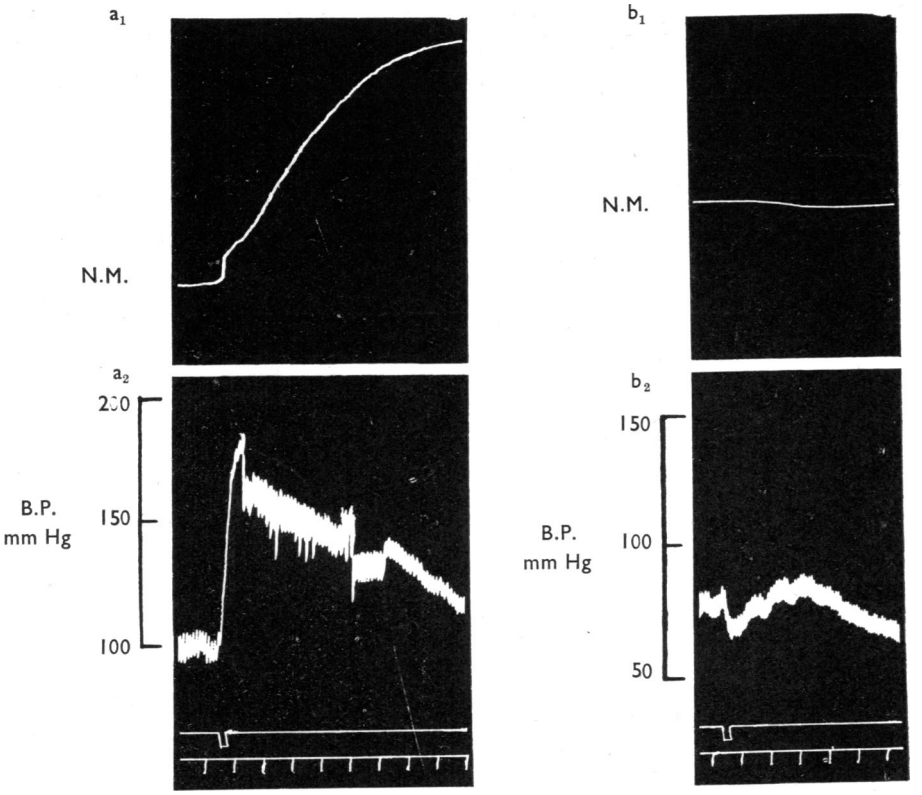

Fig. 1. Responses of nictitating membrane (upper trace) and blood pressure (lower trace) to 50 μg/kg ergotamine in cats anaesthetized with sodium pentobarbitone, 45 mg/kg. a_1, a_2, Cat without phenoxybenzamine; b_1, b_2, cat with 5 mg/kg phenoxybenzamine injected intravenously 20 min beforehand. Time signal, 1 min.

In 4 experiments where ergotamine had induced a prolonged contraction of the nictitating membrane, phenoxybenzamine (5 mg/kg) caused a gradual relaxation which reached the initial level after 5 to 10 min.

Effect of ergotamine on receptors protected by adrenaline against ergotamine blockade. Experiments were done to find out whether receptors which had been protected by adrenaline during the first exposure to ergotamine would permit the action of a second dose of ergotamine. Four experiments were made with paired preparations of rabbit aorta strip and 4 with dog retractor penis preparations. Responses to a first and second dose of ergotamine (5×10^{-8} g/ml.) were measured. Adrenaline (10^{-8} g/ml.) was also applied before and after the first dose of ergotamine, and the difference in the height of the contraction was taken to represent the degree of blockade. The control preparation of each pair was not protected by

adrenaline during the first exposure to ergotamine. Adrenaline (5×10^{-5} g/ml.) was present throughout the first exposure to ergotamine in the other preparation.

The results are presented in Table 1. In the unprotected preparations responses to adrenaline were completely or almost completely blocked by the first dose of ergotamine, and the second dose of ergotamine had no effect. However, in all

TABLE 1

EFFECT OF ADRENALINE RECEPTOR PROTECTION AGAINST ERGOTAMINE BLOCKADE ON THE STIMULANT ACTION OF ERGOTAMINE

During exposure to ergotamine, adrenaline receptors were protected by adrenaline (5×10^{-5} g/ml.) in one of two muscles from the same organ. Numerals represent mean height of contraction of the isolated muscle in mm. The values for adrenaline are the means of 3 observations before and of 2 observations after the first exposure to ergotamine. Drug concentrations are given in g/ml.

* Muscle already contracted by the protecting dose of adrenaline

	Not protected by adrenaline				Protected by adrenaline			
	Control		After ergotamine		Control		After ergotamine	
	Adrenaline 10^{-8}	Ergotamine 5×10^{-8} 1st exposure	Adrenaline 10^{-8}	Ergotamine 5×10^{-8} 2nd exposure	Adrenaline 10^{-8}	Ergotamine 5×10^{-8} 1st exposure	Adrenaline 10^{-8}	Ergotamine 5×10^{-8} 2nd exposure
Rabbit aortic strip	8	4	0	0	10	*	5	3
	12	7	0	0	18	*	7	2
	9	4	1	0	13	*	6	3
	9	3	1	0	18	*	8	5
Dog retractor penis	22	4	0	0	38	*	7	3
	32	6	0	0	46	*	11	5
	38	6	1	0	48	*	7	3
	16	5	0	0	28	*	10	6

preparations protected by adrenaline, responses to adrenaline after the first exposure to ergotamine were present although reduced, and all muscles contracted in response to the second dose of ergotamine. It is not, of course, possible to compare responses due to the first and second doses of ergotamine; the muscle at the time of the first exposure is already maximally contracted by the high concentration of adrenaline present to protect the receptors.

Effect of ergotamine on receptors protected by adrenaline against phenoxybenzamine blockade. Experiments with paired preparations were carried out to test whether protection of adrenaline receptors during exposure to phenoxybenzamine also prevented the blockade of ergotamine-induced contractions. Rabbit aorta strips were used in 6 experiments, dog retractor penis in 4 and rabbit uterus in 4. Of each pair of muscles, one was not protected and the other was protected by adrenaline (5×10^{-5} g/ml.) during exposure to phenoxybenzamine. In all but two experiments the concentration of phenoxybenzamine was 5×10^{-8} g/ml. Since this concentration gave too great a degree of blockade in the rabbit uterus preparation a concentration of 5×10^{-9} g/ml. was used in two experiments with this preparation. Responses to adrenaline (10^{-8} g/ml.) were tested before and after phenoxybenzamine to assess the degree of blockade. A subsequent dose of ergotamine (5×10^{-8} g/ml.) failed to produce contraction in unprotected muscles, but consistently caused protected muscles to contract (Table 2).

TABLE 2

EFFECT OF ADRENALINE RECEPTOR PROTECTION AGAINST PHENOXYBENZAMINE BLOCKADE ON THE STIMULANT ACTION OF ERGOTAMINE

During exposure to phenoxybenzamine, adrenaline receptors were protected by adrenaline in one of two muscles from the same organ. Numerals represent mean height of contraction of isolated muscle in mm. The values for adrenaline are the means of 3 observations before and of 2 observations after exposure to phenoxybenzamine (5×10^{-8} g/ml.). In two experiments with rabbit uterus (*) the concentration was 5×10^{-9} g/ml. Drug concentrations are given in g/ml.

	Without protection by adrenaline			Protected by adrenaline 5×10^{-5}		
		After phenoxybenzamine			After phenoxybenzamine	
	Control. Adrenaline 10^{-8}	Adrenaline 10^{-8}	Ergotamine 5×10^{-8}	Control. Adrenaline 10^{-8}	Adrenaline 10^{-8}	Ergotamine 5×10^{-8}
Rabbit	22	0	0	31	12	13
aortic	23	1	0	24	16	4
strip	32	0	0	45	21	2
	7	1	0	8	8	3
	7	0	0	8	7	2
	35	0	1	43	16	15
Dog	23	3	0	28	10	10
retractor	45	2	3	56	17	28
penis	49	0	0	46	6	6
	30	0	0	40	15	15
Rabbit	27	0	0	39	3	2
uterus	47	0	0	56	3	3
	*35	0	0	97	49	27
	*91	0	0	91	26	22

Ergometrine, which possesses the stimulant action of the ergot alkaloids but little or no blocking action, was tested in a concentration of 5×10^{-8} g/ml. in experiments with rabbit uterus preparations before and after phenoxybenzamine blockade. Responses to ergometrine were absent in unprotected preparations; in protected preparations contractions occurred. The responses to ergometrine in protected preparations were reduced to about the same degree as the responses to adrenaline. The contractions were of the same order of magnitude as those caused by ergotamine.

Protection of adrenaline receptors by ergometrine. Since the above experiments showed that ergotamine could evoke responses by acting on adrenaline receptors 12 similar experiments were carried out with ergometrine (5×10^{-5} g/ml.) instead of adrenaline to protect the receptors. Table 3 shows that ergometrine effectively protects the receptors for adrenaline, ergotamine and ergometrine.

Protection of other known receptors against phenoxybenzamine blockade. In 6 experiments protecting concentrations (5×10^{-5} g/ml.) of acetylcholine, histamine or 5-hydroxytryptamine were applied to preparations which were then treated with phenoxybenzamine. These experiments were carried out on the rabbit aorta only, since this preparation responds to all three agonists. Although the receptors for these agonists were successfully protected, ergotamine failed to elicit a contraction after phenoxybenzamine.

DISCUSSION

Ergotamine is shown to act directly on smooth muscle by combining with the receptors involved in the excitatory action of adrenaline, namely, α receptors (Ahlquist, 1948). The earlier observations of Barry (1937) and Linegar (1940) that

TABLE 3

EFFECT OF RECEPTOR PROTECTION BY ERGOMETRINE DURING ERGOTAMINE AND PHENOXYBENZAMINE BLOCKADE ON THE STIMULANT ACTION OF ERGOMETRINE, ERGOTAMINE AND ADRENALINE

Experiments were carried out as for Tables 1 and 2 except that receptors were protected by ergometrine instead of adrenaline. When phenoxybenzamine was the blocking agent, ergotamine was tested only after blockade. Drug concentrations are given in g/ml. Figures represent heights of contraction in mm. * Muscle already contracted by the protecting dose of ergometrine. ** Not tested before phenoxybenzamine

	Without protection						Protected by ergometrine 5×10^{-5}					
	Control responses			After ergotamine			Control responses			After ergotamine		
	Adrenaline 5×10^{-8}	Ergometrine 10^{-8}	Ergotamine 5×10^{-8}	Adrenaline 5×10^{-8}	Ergometrine 10^{-8}	Ergotamine 5×10^{-8}	Adrenaline 5×10^{-8}	Ergometrine 10^{-8}	Ergotamine 5×10^{-8}	Adrenaline 5×10^{-8}	Ergometrine 10^{-8}	Ergotamine 5×10^{-8}
	Ergotamine blockade (5×10^{-8})											
Rabbit aortic strip	16	4	3	0	0	0	22	5	*	15	4	7
	18	5	5	0	0	0	25	5	*	17	7	8
Dog retractor penis	31	24	17	0	0	3	28	28	*	11	4	5
	25	6	8	0	0	0	24	20	*	4	6	3
	9	11	4	0	1	0	23	4	*	12	3	3
	7	5	6	0	0	0	11	6	*	6	3	2
	Phenoxybenzamine blockade (5×10^{-8})											
Rabbit aortic strip	20	3	**	0	0	0	22	5	**	8	3	6
	24	4	**	0	0	0	53	5	**	18	3	14
Dog retractor penis	25	20	**	0	0	0	24	25	**	15	21	18
	46	53	**	0	0	0	66	60	**	28	23	17
	16	7	**	0	0	0	20	6	**	11	5	4
	21	15	**	0	0	0	30	8	**	17	7	4

ergotamine antagonizes the pressor action of subsequent doses of ergotamine are confirmed and extended to the nictitating membrane of the cat. Although these observations support the hypothesis that ergotamine acts on adrenaline receptors they could also be explained on the basis of blockade of receptors specific for ergot alkaloids. Blockade of the effects of ergometrine and ergotamine on dog uterus by dihydroergotamine, shown by Rothlin (1946), could also be explained in this way.

The hypothesis gains further support from the present experiments which show that the adrenaline antagonist phenoxybenzamine antagonizes the effects of ergotamine also. Phenoxybenzamine greatly reduced the effect of ergotamine on the nictitating membrane and on the arterial blood pressure. On isolated smooth muscle preparations doses of phenoxybenzamine which blocked the stimulant action of adrenaline also blocked the stimulant action of ergotamine. Experiments on the selective protection of receptors in smooth muscle showed that ergotamine stimulated muscles which had been protected by adrenaline against the blocking action of ergotamine or phenoxybenzamine and that ergotamine failed to excite muscles whose adrenaline receptors had not been protected. It can be concluded from these results that adrenaline and ergotamine act on the same receptors.

The results indicate that adrenaline receptors account for the greater part of the stimulant action of ergotamine. Any significant stimulant action on the receptors for acetylcholine, histamine and 5-hydroxytryptamine is excluded by the failure of ergotamine to excite muscles where only these receptors have been protected. The possibility of some minor action being mediated by other, unknown receptors cannot be completely excluded by the present experiments. It is perhaps surprising that no stimulant action on 5-hydroxytryptamine receptors could be observed, since the results of Gaddum & Hameed (1954) have shown that ergotamine must also combine with 5-hydroxytryptamine receptors in the smooth muscle of blood vessels. They observed that ergotamine antagonized the vasoconstrictor action of 5-hydroxytryptamine. Moreover, lysergic acid diethylamide, a congener of ergotamine and a competitive antagonist of 5-hydroxytryptamine in many tissues, has a pressor action in the dog similar to that of 5-hydroxytryptamine (Shaw & Woolley, 1956).

The action of ergometrine is also shown to be upon adrenaline receptors. The ability of ergometrine to combine with adrenaline receptors is established by the phenoxybenzamine blockade experiments where ergometrine stimulated muscles protected by adrenaline and where adrenaline stimulated muscles which had been protected by ergometrine. These experiments with ergometrine clarify the results presented by Konzett (1960), who found that Dibenamine antagonized the stimulant action of ergometrine at least as well as that of adrenaline on the rabbit uterus *in vivo*.

The results show that ergotamine is an adrenaline antagonist with intrinsic activity, that is, one which acts as a partial agonist (Stephenson, 1956). This view of the action of ergotamine may be applicable to other smooth muscle which is excited by adrenaline. The constrictor action on coronary vessels (Katz & Lindner, 1939) cannot yet be interpreted in this way, since it has not been fully established whether the catecholamines cause constriction or dilatation of these vessels (Schofield & Walker, 1953 ; Berne, 1958). There is, however, no clear evidence for extending

this view to smooth muscle whose activity is inhibited by adrenaline. Brown & Dale (1935) found that ergometrine had a sympathomimetic effect on rabbit ileum, but there is no convincing evidence that ergot alkaloids antagonize the action of adrenaline in this preparation. Rothlin (1946) has shown that ergotamine and dihydroergotamine prevent the relaxant action of adrenaline on isolated dog jejunum, but do not alter the tone of the preparation. It is clear that further evidence is needed to identify the receptors for ergot alkaloids in muscles where the action of adrenaline is inhibitory.

The excitatory action of ergotamine on smooth muscle from some organs cannot be explained as an action on α receptors. For example, ergotamine has been shown by Yonkman (1931) to cause miosis in the cat by direct stimulation of the sphincter of the iris, whereas adrenaline causes mydriasis and has not been shown to have an effect on the sphincter muscle. To explain these and similar observations an action of ergotamine on some other variety of receptors must be sought.

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