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BRONCHOCONSTRICTOR AGENTS AND THEIR ANTAGONISTS IN THE INTACT GUINEA-PIG

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When anaphylactic shock in the guinea-pig is elicited by inhalation of the antigen instead by injection, its onset is gradual, and its fatal outcome can be prevented by discontinuing the inhalation. This was first observed by KALLÓS and PAGEL (1937) and has been confirmed by others. The shock syndrome begins with slight slowing and deepening of respiration (in rare cases it becomes rapid and shallow) followed by a few sneezes. Then respiration slows further and is marked by deep abdominal contractions. At this stage the animal often raises its head as if fighting for more air; its nose may appear cyanotic, and, if it is not now removed from its box, it will collapse, convulse, and most probably die. If the animal is removed in time, the symptoms will continue or may even increase for a little while, but they then gradually subside. If death occurs rapidly, pathological examination invariably shows emphysema as the cause of death. As the antigen reaches the bronchi before the other organs, anaphylactic changes in the latter have not had sufficient time to develop. This shows that the syndrome described is a bronchoconstrictor shock.

KALLÓS and PAGEL (1937) have also shown that inhalation of aerosols of histamine or acetylcholine produces a very similar picture. The same holds good for 5-hydroxytryptamine (HERXHEIMER, 1953, 1955). In all these cases bronchoconstriction is the essential cause of the syndrome, as it is in anaphylactic shock produced by inhalation; wheezing can be heard over the chest of the animals when they become dyspnoic, and severe emphysema is a regular finding at necropsy. Whether any of these substances is used in the normal guinea-pig, or

the antigen in a previously sensitized guinea-pig, the speed of onset and the severity of the shock symptoms depend on the concentration of the solution the aerosol of which is inhaled. The concentration can therefore be chosen in such a way that the same exposure period (80-120 sec) is required to reach convulsion point (HERXHEIMER, 1952), whatever substance is used. This period has previously been termed preconvulsion time. The visible signs of shock are the same with all the substances mentioned. In the present experiments two further drugs were used as bronchoconstrictor substances, namely nicotine and " Furmethide ".

The experiments were designed to test, in the intact guinea-pig, the effect of various drugs which, in the isolated organ, have been proved to be antagonists of histamine, acetylcholine, nicotine, muscarine or 5-hydroxytryptamine. Their antagonistic effect against these bronchoconstrictor agents was compared with their effect previously obtained in anaphylactic shock.

METHOD

The aerosol method used in these experiments was the microshock method which has been previously described (HERXHEIMER, 1952). The aerosol was produced by leading compressed air at constant pressure through a commercial nebulizer. From the nebulizer the aerosol entered a box (an inverted aquarium resting on a rubber sponge mat) and was allowed to escape from the bottom where the box rested on its rubber base. When the box was filled with the aerosol, equilibrium of density was reached within one minute. The box was then lifted at one end and the animals quickly put in.

The bronchoconstrictor substances used were histamine acid phosphate 0.5 % (H), acetylcholine bromide 3.125 % (Ach), methacholine chloride 0.25 % (M), nicotine sulphate 4 % (N), 5-hydroxytryptamine creatinine sulphate 1 % (HT) and 5-methylfurfuryltrimethylammonium iodide 0.25 % (F). This substance (" Furmethide ") has been described by ING, KORDIK and TUDOR WILLIAMS (1952) and has a muscarine-like effect. The antagonists used were hexamethonium bromide, atropine sulphate, propantheline, chlorpromazine, papaverine sulphate, lysergic acid diethylamide (LSD), adrenaline tartrate, mepyramine, aminophylline and cocaine hydrochloride. The antagonists were given either by intramuscular injection 15-60 min. before inhalation of the aerosol (mepyramine, LSD, atropine and adrenaline), or intraperitoneally 20 min.

before (papaverine, propantheline, chlorpromazine and aminophylline). Cocaine was administered as an aerosol in a 0.25 % or 0.5 % solution for periods ranging from 5 to 25 min immediately before shock exposure. The chloride of hexamethonium was used in some experiments until it was clear that the effects of the chloride and the bromide were identical. With most of the antagonists a wide range of dosage was used (TABLES I-III). The antagonistic action of a drug was expressed as the " percentage protection " (HERXHEIMER, ARMITAGE and ROSA, 1952), calculated from the formula $(1 - \frac{C}{T} 100)$, when C is the preconvulsion time of the controls and T the preconvulsion time with the antagonist. All the antagonists have also been tested in anaphylactic shock. Guinea-pigs actively sensitized to egg albumin were used and shocked by the microshock method as described. Some of the experiments with antagonists in anaphylactic shock have already been described (ARMITAGE et al., 1952, HERXHEIMER & ROSA, 1953); others are described in the present series.

TABLE I

	Atropine mg/kg					Propantheline mg/kg					Hexamethonium mg/kg						
	.013	.065	.13	.32	1.28	.1	.2	1.0	2.0	10.0	.01	.1	.25	1.0	5.0	10.0	20.0
M	13	31	76	94		24	83		96	96				33	58	56	25(2)
Ach	49	71	81	96			36			81				44		50	54
F	0	69	54	85			59		85						28	37	27*(2)
N			40	66	85		0	53		63	0	53	78	89	98		93
H			30	38	54		54	63		75				41	55	53	7*(2)
HT			12*	37	58			60		52						26*	44
Ana				15	59			41(1)	46	40						38	36

The figures represent the percentage protection given by the drug against the bronchoconstrictor substance. M = Methacholine, Ach = Acetylcholine, F = Furmethide, N = Nicotine, H = Histamine, HT = 5Hydroxytryptamine, Ana = anaphyl. shock. The figures given have been found statistically significant with the exception of those with an asterisk.

(1) 0.5 mg/kg propantheline.

(2) Animals unstable on their feet.

Results

Nicotine (N) and Furmethide (F) had a similar effect on the respiration as had histamine (H) and the other bronchoconstrictor substances. The initial symptoms and the shock syndrome were the same, with the exception that the nicotine aerosol seemed to cause much more sneezing and scratching of the nose than the other aerosols. A fatal outcome of the shock occurred from time to time with all the substances when, by an error of judgment, an animal was left in the box a few seconds too long. With HT this was a rare exception, as tachyphylaxis with this substance develops quickly (Herxheimer, 1955).

When large doses of hexamethonium (10 mg and 20 mg/kg) were given, the animals often tottered and found it difficult to keep on their feet. They seemed to die more easily when they were shocked than in experiments with lower doses or with other antagonists, if they were left in the box a few seconds too long.

The effect of the antagonists is shown in the Tables I-III. Three different groups of antagonists can be separated : (a) Atropine, propantheline, hexamethonium, cocaine and adrenaline. These drugs antagonize all or most shock producing agents, but one particular agent more than the others. Atropine and propantheline strongly antagonize Ach,

Table II

	Adrenaline mg/kg							Cocaine min. expos.				Aminophyll. mg/kg			Chlorpromazine mg/kg			
	.01	.02	.04	.05	.1	.2	.3	1.5	5.0	12	25	25	50	100	3	5	10	20
M				20*	13*	33				38	42	33*	80			14*	39*	
Ach				0	39					32	45		72			43	71	
F					32					23*			62			14*	72	
N	36	37	59		85			27	86	91			49	86		36	52	
H				19*		69				52		0	64	80	0		67	
HT						33				33	4		85			48	55	
Ana	29					57				49			56(1)	100		56	49	

Explanation see Table I.
(1) 40 mg/kg Aminophylline.

TABLE III

	Papaverine mg kg					Mepyramine mg kg					LSD mg kg									
	20	40	50	60	80	.001	.01	1.0	3.0	6.0	.005	.01	.02	.05	.067	.075	.08	.1	.4	
M	17*	31		40				15*	24	0			40	−11	−17		−5			
Ach		44						30	39							−37		−32		
F		31		26				54	28					12			−28			
N	32	52			76			22*	17*				10		−23	−30				
H			50			0	77					62		−53						
HT		0	29*	80				25*	21*		11	30	60	70					68	85
Ana		0	0	0				15*	64	78	58		0	0					0	

Explanation see Table I.

M and F, whilst N is less affected, and H and HT still less. Hexamethonium antagonizes N much more strongly than the other bronchoconstrictors. The same holds good for cocaine and adrenaline, but their action is weaker than that of hexamethonium.

b) Aminophylline, papaverine and chlorpromazine. These drugs antagonize all shock producing agents about equally well, and only in large doses.

c) Mepyramine and LSD. These drugs antagonize strongly only one agent (H and HT) and the others weakly or not at all. LSD, in addition, increases the shock symptoms of almost all shock producing agents (as can be seen from the „ negative " protection figures in Table III) except those of HT which it antagonizes.

Discussion

Mepyramine and LSD. The almost exclusive antagonism of mepyramine to H confirms the findings of Schild (1947) and others, according to which this drug, in contrast to other antihistamines, has very little anti-Ach affect and is an almost " pure " antihistamine. The strong antagonism of LSD to HT has been described before (Herxheimer, 1955). No reason for the increase of the shock symptoms by LSD can be suggested.

Hexamethonium. The action of this substance as a highly specific ganglion blocker is not disputed. In the present experiments it had a strong antagonistic effect to N, which is believed to have an almost exclusive effect on the ganglion; it blocks it in smaller doses than any other substance. This confirms the ganglion blocking action of hexamethonium. If it has no other action than blocking the ganglion, its less strong antagonism to Ach and M is compatible with this view. The actions of these substances can be separated into a nicotinic (on the ganglion) and a muscarinic action (on the muscle itself). If hexamethonium blocks only the former, it would be understandable that its protection against Ach and M is smaller than that against N, as it would block only the nicotinic part of the Ach effect. If this is the case, the muscarinic part of this effect should not be influenced by hexamethonium. The present experiments indicate that the effect of hexamethonium on F is indeed weak.

These observations confirm, or, at least, are compatible with a pure ganglionic action of hexamethonium. Its marked protective action in H shock suggests a ganglionic action of H. Such an action has been observed recently by Trendelenburg (1954). Ambache and Lessin (1955) have described a " neuronal " action of H in the rabbit gut. Although hexamethonium had a constant and reproducible effect in the present experiments in small and medium doses, large doses of 10 mg and 20 mg/kg sometimes did not produce an increased effect, and, in a few cases, the effect decreased. With all other antagonists the protective effect regularly increased with dosage. It looks, therefore, as if large doses of hexamethonium bring other factors into action which impair the protective effect. As the animals appeared unstable on their feet with such doses, it may be suspected that the hypotensive effect of the hexamethonium is an indirect cause of this impairment, but a direct bronchoconstrictor effect of the larger doses may also be the cause.

Atropine and propantheline. It appears doubtful whether the whole antagonistic action of atropine takes place at the postganglionic synapse. If this were the case, one would expect it to antagonize equally strongly N, Ach and F, as it would not only prevent all muscarinic action on the muscle, but would also block, at this point, impulses arriving through the postganglionic fibres. Its action against N is however much weaker than that against Ach and F. This is difficult to explain with a purely musculotropic action of atropine or its action on the postganglionic synapse. It may be that atropine acts particularly strongly against Ach applied from outside, and more strongly than against N. There is also the possibility of a direct ganglionic action of atropine (MARRAZZI, 1938, and others). The antagonism of atropine to H could be explained by a ganglion blocking action, particularly as the antagonism of hexamethonium against H makes an action of H on the ganglion probable. Atropine also has a direct antihistaminic action (SCHILD, 1947).

Cocaine. Its specific antagonism to N can be explained satisfactorily by the paralysing effect of cocaine on the postganglionic nerve fibre. If N acts by ganglionic stimulation, the paralysis of the postganglionic nerve fibre would prevent the stimulus from reaching the peripheral organ. The small degree of the cocaine protection against the other substances in the present experiments should not be unduly stressed, as the longer administration of cocaine by aerosol makes the dosage uncertain and has side effects which possibly interfere with its protective action.

Adrenaline. As this substance has, in most species, a strong effect against bronchial obstruction, and as its action in anaphylactic shock is also pronounced, a strong protective effect was expected in the present experiments. It was observed, however, only against N. Against Ach, M and F doses near the toxic level were almost ineffective, whilst they gave good protection against H and weak protection against HT. An explanation of this surprising result cannot be given.

Aminophylline, papaverine, chlorpromazine. The common characteristic of these substances is that they act as antagonists whatever the bronchoconstrictor agent, and with about the same intensity. Aminophylline is more effective than the others, and comparatively high doses are required of all three. Aminophylline and papaverine are known to be bronchodilators, and we may assume that their antagonistic actions take place in the muscle itself. The reason for the antagonistic action

of chlorpromazine is probably different. The weak antihistaminic and anticholinergic properties it has shown in the present experiments, have been seen by other authors, but no direct bronchodilator effect has been described.

Anaphylactic shock. All antagonists used in the present series except LSD and papaverine also protect against anaphylactic shock, but to different degrees. Both adrenaline and mepyramine give pronounced protection in anaphylaxis, and, at the same time, they protect either more against H than against any other bronchoconstrictor, or exclusively against it. This points to the dominant part H may play in the production of the symptoms of anaphylaxis. The reason for the much weaker effect of mepyramine against anaphylactic shock than against H may well lie, as DALE has suggested, in the different accessibility of the H. It may be more easier for the antihistamine to antagonize H if it is applied from outside by aerosol than if it is liberated from within the cells. It is difficult to follow the suggestion of HAWKINS (1955) that a bronchoconstrictor effect of the antihistamines in high concentration might be the cause of their comparatively weak action in anaphylactic shock. If this were the case, we should expect the antagonism of mepyramine to H to decrease with the large doses which are supposed to have a bronchoconstrictor effect. There is no evidence for this.

The other antagonists do not provide stronger protection against the various bronchoconstrictor substances than they provide against anaphylaxis. Atropine and hexamethonium protect less against anaphylaxis than against Ach and N respectively. From the effect of adrenaline and mepyramine it may be concluded that their antagonistic action in anaphylaxis is based on their antagonistic action to H. It is remarkable that papaverine, which protects moderately well against H, has no effect in anaphylaxis.

The present experiments do not contribute much to the explanation of the nature of anaphylactic shock. They confirm that H plays a dominant part in it, and they show that the nervous elements of the bronchial mucosa are also involved. As we do not know in which site H and other endogenous bronchoconstrictor substances are formed and act, it does not seem permissible to draw further conclusions from these experiments in which the bronchoconstrictor agents were applied from outside.

Summary

1. The antagonistic effect of atropine, propantheline, hexamethonium, adrenaline, papaverine, aminophylline, chlorpromazine, mepyramine, cocaine and LSD against the bronchoconstrictor action caused in the intact guinea-pig by the inhalation of histamine, acetylcholine, methacholine, nicotine, " Furmethide " and 5-hydroxytryptamine aerosols has been investigated.

2. Atropine, propantheline and hexamethonium antagonise all these bronchoconstrictors. The first two act more strongly against the acetylcholine group, whilst hexamethonium, cocaine and adrenaline antagonize nicotine more strongly than any other substance. Mepyramine and LSD antagonize exclusively histamine and 5-hydroxytryptamine respectively. Aminophylline, papaverine and chlorpromazine antagonize all bronchoconstrictors equally well.

3. The antagonism of hexamethonium against histamine suggests that ganglionic mediation is involved in the action of histamine. Its action against anaphylactic shock suggests that the nervous elements of the bronchial membrane play some part in the symptoms of anaphylaxis.

4. LSD increased the bronchoconstrictor action of all substances except that of 5-hydroxytryptamine.

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