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The effects of acetates of aliphatic alcohols on the cholinergic nerve structures and the acetylcholine receptor of the guinea-pig ileum

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Isoamyl acetate, n-butyl acetate, n-amyl acetate and n-propyl acetate produced contractions of the isolated ileum of the guinea-pig. These were inhibited by atropine, procaine and by cooling. These acetates and also s-butyl, t-butyl and n-octyl acetate behaved as inhibitors of acetylcholine at 14 ± 1°. The acetylcholine content in the organ bath fluid increased after 60 min incubation of the ileum with isoamyl acetate. The results indicated that the agonistic acetates produced contraction of ileum through the liberation of acetylcholine from the cholinergic nerve-endings. All the acetates behaved as inhibitors of acetylcholine when they combined with the acetylcholine receptor on the muscle.

IT was recently reported (Takagi, Takayanagi, Ishida & Moritoki, 1965, Takagi & Takayanagi, 1966) that two mechanisms could be responsible for the acetylcholine liberation from the cholinergic nerve plexus of the guinea-pig ileum. One was liberation by 5-hydroxytryptamine and picric acid (Takagi & Takayanagi, 1962, 1965) and was blocked by morphine and strychnine; the other was effected by phenyl acetate and was resistant to the action of morphine and strychnine. We have now examined the modes of action of acetates of aliphatic alcohols on the isolated ileum of the guinea-pig.

Methods and materials

The experiments were made on 3 to 4 cm strips of male guinea-pig ileum suspended in Tyrode solution, gassed with oxygen 95% and carbon dioxide 5%. The responses of the gut were recorded on a smoked paper. The bath of 40 ml capacity was usually maintained at 32°. In some experiments the temperature of the bath fluid was lowered to 14 ± 1° for 1 to 1½ hr. The fundamental methods of the agonist-antagonist techniques are in principle the same as those reported by Brownlee and his colleagues (Harry, 1962; Brownlee & Johnson, 1963).

Some experiments were made on the frog rectus abdominis muscle to test the effects of isoamyl acetate on acetylcholine-induced contractions.

The acetylcholine released from the isolated ileum was estimated by the method of Schaumann (1957). Ilea (20 g) from three guinea-pigs were cut into pieces 1 to 1·5 cm in length which were mixed and divided into two lots (each of 10 g). Tyrode solution (40 ml) which contained physostigmine salicylate (3×10^{-5} M) was added to one lot which was then incubated at 32° for 1 hr, during which isoamyl acetate (2×10^{-3} M) was added at zero time and then after 15, 30 and 45 min incubation (total 8×10^{-3} M). The control group was similarly treated except that Tyrode was added instead of isoamyl acetate. After centrifuging the

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incubation mixture, the acetylcholine content of the supernatant was assayed on the excised rectus abdominis muscle of the frog treated with physostigmine salicylate (3×10^{-5} M). Results are the means of at least 8 experiments.

DRUGS

Drugs under test. n-Amyl acetate, isoamyl acetate, n-butyl acetate, s-butyl acetate, t-butyl acetate, n-octyl acetate, n-propyl acetate.

Agonists. Acetylcholine chloride, dimethylaminoethyl acetate hydrochloride, 5-hydroxytryptamine creatinine sulphate, nicotine bitartrate, phenyl acetate, picric acid.

Antagonists. Atropine sulphate, morphine hydrochloride, procaine hydrochloride.

Results

EXPERIMENTS ON THE ISOLATED ILEUM

Isoamyl, n-butyl, n-propyl and n-amyl acetate contracted the isolated ileum, but n-butyl, n-propyl and n-amyl acetate were only partial agonists (Table 1). The contractions were inhibited by treatment for 5 min with

TABLE 1. INTRINSIC ACTIVITIES (i.a.) AND AFFINITIES (pD_2 OR pA_2) OF ACETATES OF ALIPHATIC ALCOHOLS

	i.a.	pD_2	pA_2 (ACh)	pA_2 (IA)	pA_2 (ACh)	pD_2
Temperature (°C)	32	32	32	32	14 ± 1	14 ± 1
n-Octyl acetate	0	—	3·65	3·58	3·76	—
n-Amyl acetate	0·38	2·82	2·99	2·89	3·07	—
Isoamyl acetate (IA)	0·92	3·52	—	—	2·60	—
n-Butyl acetate	0·62	3·20	2·56	2·66	2·81	—
s-Butyl acetate	0·1	—	3·00	2·93	2·98	—
t-Butyl acetate	0	—	2·81	2·78	2·83	—
n-Propyl acetate	0·32	2·91	3·24	3·23	3·19	—
Isopropyl acetate	0	—	3·20	3·08	3·19	—
Acetylcholine (ACh)	1·00	7·12	—	—	—	7·22
Dimethylaminoethyl acetate	1·00	5·25	—	—	—	5·26
Atropine	0	—	8·32	8·28	8·20	—

The pA_2 values were obtained as the competitive inhibitory activities against the agonist in the parentheses.
i.a. = maximum response by test agonist/maximum response by acetylcholine.

atropine (2×10^{-8} M), for 60 min with procaine (2×10^{-4} M), or by cooling the ileum to 14 ± 1° for 1 to 1½ hr (Fig. 1) but not by 3 min treatment with morphine (10^{-5} M). Cooling and treatment with procaine also abolished the responses induced by 5-hydroxytryptamine (5-HT), picric acid, nicotine and phenyl acetate which have been shown to release acetylcholine from the cholinergic nerve plexus in the ileum, but the responses to acetylcholine were unaffected by this treatment (Fig. 1). The acetates which did not contract the ileum displaced the dose-response curves of acetylcholine and isoamyl acetate towards higher concentrations in a parallel manner indicating a competitive inhibitory action against acetylcholine and isoamyl acetate. Synergism between t-butyl acetate or n-propyl acetate and atropine was examined. The percent maximum

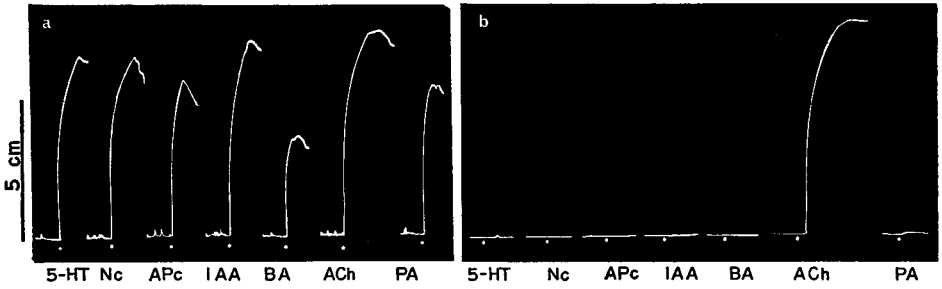

FIG. 1. Effect of cooling guinea-pig ileum on the responses produced by 5-hydroxytryptamine (5-HT), picric acid (PA), nicotine (Nc), phenyl acetate (APc), isoamyl acetate (IAA), n-butyl acetate (BA) and acetylcholine (ACh). (a) responses at 32°, (b) responses at 14 ± 1°. 5-HT, $3{\cdot}0 \times 10^{-5}$ M; Nc, $5{\cdot}0 \times 10^{-5}$ M; APc, $3{\cdot}0 \times 10^{-4}$ M; IAA, $1{\cdot}0 \times 10^{-3}$ M; BA, $2{\cdot}0 \times 10^{-3}$ M; ACh, $1{\cdot}0 \times 10^{-7}$ M; PA, $3{\cdot}0 \times 10^{-4}$ M. Note that only the response produced by acetylcholine was not inhibited by cooling.

contractions of the ileum to acetylcholine plotted against the molar concentrations of acetylcholine (log scale) are given in Fig. 2. Curve a is the control dose-response curve to acetylcholine, curve b is the dose-response curve to acetylcholine in the presence of t-butyl acetate ($1{\cdot}7 \times 10^{-3}$ M); curve c is the dose-response curve in the presence of atropine (2×10^{-8} M); curve d is the dose-response curve to acetylcholine in the presence of atropine (2×10^{-8} M) and t-butyl acetate ($1{\cdot}9 \times 10^{-3}$ M). It can be seen from Fig. 2 that the distance between curves a (acetyl-

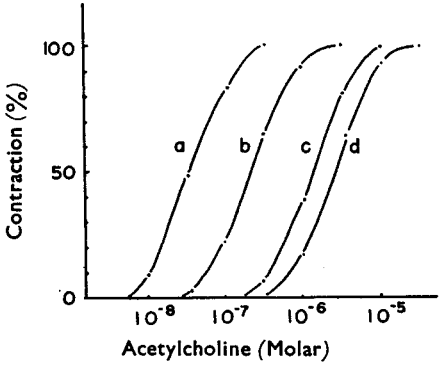

FIG. 2. Synergism between t-butyl acetate and atropine. (a) acetylcholine alone, (b) acetylcholine with t-butyl acetate $1{\cdot}7 \times 10^{-3}$ M, (c) acetylcholine with atropine 2×10^{-8} M, (d) acetylcholine with atropine and t-butyl acetate $1{\cdot}7 \times 10^{-3}$ M.

choline alone) and b (acetylcholine with t-butyl acetate, $1{\cdot}7 \times 10^{-3}$ M) was less than that between curves c (acetylcholine with atropine, 2×10^{-8}M) and d (acetylcholine with atropine, 2×10^{-8} M and t-butyl acetate, $1{\cdot}7 \times 10^{-3}$ M). Moreover, curve d was quantitatively identical to the

theoretical value calculated from equation 1, an equation for additive antagonism (Takagi & Takayanagi, 1964).

$$\frac{y}{y'} = \frac{A/K_A}{1 + A/K_A + B/K_B + C/K_C} \quad \ldots \quad \ldots \quad (1)$$

where: y' = maximum response, y = response induced by agonist A in the presence of antagonists B and C, and K_A, K_B, K_C are dissociation constants of the corresponding drugs (A, B, C).

According to Takagi & Takayanagi (1964), for potentiation between t-butyl acetate and atropine to occur the distance between the curves a and b must be equal to that between the curves c and d. The present result indicated that the synergism between t-butyl acetate and atropine was additive synergism and not potentiation. A similar result was obtained with atropine and n-propyl acetate. These results suggested that the inhibitory acetates combined with the acetylcholine receptors to inhibit the contraction due to acetylcholine. The effects of cooling the ileum on the actions of acetylcholine and dimethylaminoethyl acetate, both of which combine directly with the acetylcholine receptor to contract the muscle were further examined. The affinities of the agonists were expressed as the pD_2 values (negative logarithm of the concentration which produced 50% of the maximal contraction of muscle); the competitive inhibitory activities were expressed as the pA_2 values which were calculated from shift of a dose-response curve of an agonist using the table of van Rossum (1963). The pA_2 value of atropine against acetylcholine and the pD_2 values of acetylcholine and dimethylaminoethyl acetate were little affected by cooling the ileum to $14 \pm 1°$, so using acetylcholine as an agonist, the pA_2 values of the acetates tested at a temperature of $14 \pm 1°$ must represent their affinity for the acetylcholine receptor on the smooth muscle. The intrinsic activities and affinities (pD_2 or pA_2) of all the acetates of aliphatic alcohols tested on the isolated ileum of the guinea-pig are summarised in Table 1. These results suggest that the agonistic acetates, especially isoamyl acetate and n-butyl acetate, contract the ileum through the liberation of acetylcholine from its cholinergic innervation and that when these acetates combine with the acetylcholine receptor, they behave as competitive inhibitors of acetylcholine.

INCREASE OF THE OUTPUTS OF ACETYLCHOLINE FROM THE GUINEA-PIG ILEUM

The mean ($\pm$ s.e.) of the spontaneous outputs of acetylcholine from the ileum (control group) was $3{\cdot}2 \pm 0{\cdot}8$ μg/g tissue/hr; the mean ($\pm$ s.e.) of the amount of acetylcholine liberated from the ileum (test group) treated with isoamyl acetate (8×10^{-3} M) was $6{\cdot}3 \pm 1{\cdot}2$ μg/g tissue/hr. Thus isoamyl acetate accelerated the release of acetylcholine from the ileum.

EXPERIMENTS ON THE FROG RECTUS ABDOMINIS MUSCLE

Isoamyl acetate in concentrations up to 4×10^{-3} M did not contract the frog rectus abdominis muscle. After incubation of the muscle with isoamyl acetate (4×10^{-3} M) for 40 min, the affinity (pD_2) of acetylcholine

was not altered. This suggests that isoamyl acetate has only weak anticholinesterase activity, if any.

Discussion

Burgen (1965) has recently reported that 3,3-dimethylbutyl acetate and isoamyl acetate induced contraction of the guinea-pig ileum. On the other hand Takagi, Takayanagi & Fujie (1958) reported that isoamyl acetate did not contract the longitudinal muscle of the mouse ileum or the frog rectus abdominis muscle but behaved as a competitive and non-competitive antagonist of acetylcholine. This leads us to inquire into the possibility that the contractions of the guinea-pig ileum caused by isoamyl acetate might result from the activation of a receptor mechanism absent in the frog rectus. We have found evidence in favour of this possibility since the experiments described above have revealed that isoamyl acetate causes an increase in the acetylcholine output from the guinea-pig ileum. The fact that the effects of acetates of aliphatic alcohols, especially isoamyl acetate and n-butyl acetate were inhibited by atropine and procaine and by cooling, suggests that the site of action of these agonistic acetates must be in the nerve structures.

Two mechanisms have been postulated by Takagi & Takayanagi (1966) and Takagi, Takayanagi, Ishida & Moritoki (1965) for the liberation of acetylcholine from the guinea-pig ileum: the first mechanism is activated by electrical stimulation at low frequencies and by 5-HT and picric acid and is depressed by morphine; the second is activated by higher frequencies of electrical stimulation and by the action of phenyl acetate and is resistant to the inhibitory action of morphine. In the present experiments the responses induced by the agonistic acetates were not inhibited by 3 min treatment with morphine, so that acetylcholine released by them may result from the activation of the second mechanism.

It was recently reported by Johnson (1964) and Brownlee & Johnson (1965) that 5-HT and dimethylphenylpiperazinium activated different nerve pathways in the cholinergic nerve plexus of the guinea-pig ileum. Takagi, Takayanagi, Irikura & others (1965) have observed that spontaneous movements of ileum removed from guinea-pigs exhibiting morphine withdrawal symptoms were greatly increased and this increase of the spontaneous activity could be inhibited by morphine but not by hexamethonium. From this and from the further observations on the ileum from guinea-pigs exhibiting withdrawal symptoms that the effects of 5-HT were also greatly increased but those of nicotine were not altered, Takagi, Takayanagi, Irikura & others (1965) postulated that the increase of the spontaneous movements might be explained by the excitation of a nerve pathway activated by 5-HT but not by the nerve pathway activated by nicotine. This observation supports Johnson's conclusion on the nerve pathways.

The two nerve pathways proposed by Johnson (1964) would seem to constitute our acetylcholine liberation mechanism which is depressed by morphine.

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