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Potentiation by sulphydryl agents of the responses of guinea-pig isolated ileum to various agonists

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Sulphydryl agents (dithiothreitol, mercaptoethanol, monothioglycerol, cysteine, glutathione) increase non-specifically the potency of various agonists (acetylcholine, 5-hydroxytryptamine, nicotine, bradykinin, prostaglandin E_2) on the guinea-pig ileum. These effects are of non-cholinergic origin and are not related to prostaglandin synthesis. Sulphydryl agents act directly on the smooth muscle cells of the guinea-pig ileum most probably by reducing disulphide bonds located on the membrane surface and by this mechanism modulate the muscular contractile activity.

We have previously demonstrated (Famaey et al 1976) that D-penicillamine (dimethylcysteine), a drug known for its antirheumatic properties (Arrigoni-Martelli & Bramm 1975) is able to increase the responses of the guinea-pig isolated ileum to various agonists.

This contrasts with the inhibitory effects of anti-inflammatory drugs on the same preparation (Famaey et al 1977, 1979) and has been related to the ability of D-penicillamine to increase prostaglandin (PG) synthesis in other tissues (Maddox 1973).

On the other hand, it has been shown that cysteine and dimercaptopropanol potentiate the contractions produced by bradykinin in the guinea-pig ileum and this effect has been related to inhibition of kininase present in the tissue (Ferreira et al 1962; Picarelli et al 1962). Potter & Walaszek (1972), however, demonstrated that cysteine can augment contractions of the guinea-pig ileum elicited by a variety of spasmogens and exerts its effect by facilitating release of acetylcholine from nerve endings.

The action of dithiothreitol (DTT) on guinea-pig ileum has also received attention. Glover (1979) showed that this agent, which reduces disulphide bonds to sulphydryl groups, potentiates the contractile responses of the guinea-pig ileum to histamine, by stimulation of H_1 receptors. This selective increase in sensitivity to histamine by DTT treatment had been previously demonstrated in a vascular preparation in-vitro (Fleisch et al 1973) and we have shown that various sulphydryl agents potentiate the blood pressure-lowering effect of histamine in the anaesthetized dog (Fontaine et al 1979).

On the other hand, Watson & Iversen (1982) recently demonstrated that DTT non-specifically potentiates the actions of spasmogens on the guinea-pig ileum.

In view of these contradictory results we have systematically compared the effects of five sulphydryl compounds (dithiothreitol, mercaptoethanol, monothioglycerol, cysteine, glutathione) on the responses of the guinea-pig ileum to various agonists (acetylcholine, histamine, 5-hydroxytryptamine, nicotine, bradykinin, PGE_2). To see if the effects exerted by these drugs can be related to neuronal release of acetylcholine or to prostaglandin synthesis, several preparations have been preincubated with atropine, tetrodotoxin or indomethacin.

METHODS

Adult guinea-pigs of either sex (300–400 g) were stunned and bled. Segments of ileum (4 cm) at least 10 cm from the caecum were set up in Krebs-Henseleit solution (composition mg ml^{-1}: KCl 0·35; NaCl 6·92; $CaCl_2.2\ H_2O$ 0·37; $NaHCO_3$ 2·1; KH_2PO_4 0·16; $MgSO_4.7\ H_2O$ 0·29; glucose 1·0) maintained at 37 °C and gassed with a mixture of 5% CO_2 and 95% O_2.

Submaximal contractions were induced by acetylcholine (ACh) 4 ng ml^{-1}, histamine 10 ng ml^{-1}, 5-hydroxytryptamine (5-HT) 30 ng ml^{-1}, nicotine 1 μg ml^{-1}, bradykinin 6 ng ml^{-1} or PGE_2 3·5 ng ml^{-1} every 3 or 6 min and were recorded by an isotonic lever (five-fold magnification). After at least three reproducible contractions to one of these agonists, a sulphydryl agent was added to the Krebs solution and was left in the bath for 12 min, contractions of the ileum being elicited every 3 min (with ACh, histamine, bradykinin) or 6 min (with 5-HT, nicotine or PGE_2). The ileum was again challenged with the same agonist after

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washing out the sulphydryl agent from the bath. The results (mean ± s.e. mean, 4–9 experiments) were expressed as a percentage of the average height of the 3 last pre-thiol responses ('control responses') and were analysed by Student's *t*-test for paired data.

In some experiments atropine (100 ng ml^{-1}), tetrodotoxin (100 ng ml^{-1}), or indomethacin (0·63 μg ml^{-1}) were added to the Krebs solution from the beginning of the experiment (n = 4 or 5 for each antagonist).

Drugs

Acetylcholine hydrochloride (Roche), atropine sulphate (Merck), bradykinin triacetate (Aldrich), DL-cysteine hydrochloride (Sigma), 1,4-dithiothreitol (Calbiochem), glutathione (Sigma), histamine hydrochloride (Fluka), 5-hydroxytryptamine hydrogenmaleinate (Fluka), indomethacin (Merck Sharp & Dohme), 2-mercaptoethanol (Sigma), α-monothioglycerol (Sigma), nicotine sulphate (BDH), prostaglandin E_2 (Upjohn), tetrodotoxin crystalline 3 × (Calbiochem).

RESULTS

Effects of dithiothreitol (Table 1)

Incubation of guinea-pig ileum with dithiothreitol (DTT) 10 or 40 μg ml^{-1} increased significantly the potency of all agonists tested by 20–60%. However, the responses induced by nicotine were significantly decreased after incubation with 40 μg ml^{-1} DTT. The sensitization was immediate but augmented with time and became stable after 12 min of contact. DTT, 40 μg ml^{-1}, appeared in most cases to cause more sensitization than 10 μg ml^{-1} but this difference was not statistically significant except with bradykinin.

Twelve min after washing out DTT, the responses to 5-HT, bradykinin and PGE_2 returned to control values, while the responses to ACh, histamine and nicotine remained higher.

Effects of mercaptoethanol and monothioglycerol (Table 1)

Incubation of guinea-pig ileum with mercaptoethanol or monothioglycerol (10 μg ml^{-1}) was found to increase the potency of all agonists tested by 15–50%. After incubation with 40 μg ml^{-1} of the sulphydryl agent, the responses of the ileum were not higher and no statistically significant difference could be found between the effects of 10 and 40 μg ml^{-1} of mercaptoethanol or monothioglycerol.

The sensitizing effects of these two sulphydryl agents were immediate but those of mercaptoethanol augmented generally with time and became stable after 12 min of contact.

Table 1. The effects of dithiothreitol, mercaptoethanol, monothioglycerol, cysteine, glutathione 10 and 40 μg ml^{-1} on guinea-pig ileum submaximal contractions induced by acetylcholine (ACh), histamine (Hist), 5-hydroxytryptamine (5-HT), nicotine (Nic), bradykinin (Bk) and prostaglandin E_2 (PGE_2). Results, from 12 min of incubation with the sulphydryl agent are expressed in percentage of control submaximal contractions (in the absence of the sulphydryl agent). Mean ± standard error of the mean values were calculated (n). Results in parentheses are those obtained 12 min after washing out the sulphydryl agent from the bath. Statistical analysis was by Student's *t*-test for paired data.

Sulphydryl agent (μg ml^{-1})	ACh	Hist	5-HT	Nic	Bk	PGE_2
Dithiothreitol 10	134 ± 2*** (6)	128 ± 7* (7)	119 ± 8* (9)	128 ± 8* (6)	119 ± 5* (8)	142 ± 16* (4)
	(129 ± 3)	(116 ± 12)	(81 ± 12)	(142 ± 19)	(83 ± 10)	(112 ± 7)
40	150 ± 13** (6)	138 ± 10* (6)	123 ± 5** (7)	48 ± 15* (6)	162 ± 13** (7)	137 ± 10** (6)
	(155 ± 14)	(146 ± 12)	(82 ± 9)	(70 ± 21)	(108 ± 20)	(110 ± 11)
Mercaptoethanol 10	119 ± 5* (8)	147 ± 14* (7)	134 ± 11* (7)	128 ± 11* (5)	114 ± 3* (6)	137 ± 6* (7)
	(113 ± 11)	(140 ± 21)	(114 ± 8)	(125 ± 13)	(108 ± 12)	(110 ± 10)
40	117 ± 4* (8)	126 ± 8* (9)	143 ± 18* (8)	136 ± 10* (6)	118 ± 6* (7)	135 ± 10* (7)
	(120 ± 13)	(166 ± 10)	(116 ± 12)	(121 ± 16)	(75 ± 11)	(103 ± 11)
Monothioglycerol 10	125 ± 7* (6)	128 ± 6** (8)	129 ± 9** (8)	126 ± 8* (8)	131 ± 7** (8)	122 ± 4* (7)
	(115 ± 5)	(126 ± 10)	(128 ± 12)	(112 ± 7)	(92 ± 15)	(98 ± 7)
40	127 ± 6** (8)	122 ± 6** (8)	120 ± 6* (8)	130 ± 7* (8)	151 ± 16* (8)	139 ± 12* (6)
	(119 ± 5)	(105 ± 6)	(96 ± 7)	(122 ± 10)	(102 ± 13)	(99 ± 19)
Cysteine 10	129 ± 11* (6)	133 ± 7*** (7)	138 ± 14* (7)	131 ± 8* (6)	155 ± 21* (4)	106 ± 6 (10) NS
	(125 ± 23)	(102 ± 11)	(111 ± 11)	(124 ± 12)	(113 ± 15)	(81 ± 11)
40	124 ± 5** (7)	124 ± 11* (8)	159 ± 12** (6)	116 ± 4** (7)	157 ± 5*** (6)	108 ± 5 (10) NS
	(115 ± 16)	(99 ± 5)	(109 ± 14)	(95 ± 11)	(68 ± 8)	(85 ± 7)
Glutathione 10	126 ± 7** (8)	140 ± 14* (6)	125 ± 8** (6)	120 ± 10 (10) NS	130 ± 9* (6)	134 ± 11* (5)
	(83 ± 13)	(119 ± 15)	(105 ± 18)	(140 ± 20)	(98 ± 17)	(120 ± 10)
40	137 ± 7*** (7)	135 ± 10*** (7)	129 ± 9* (7)	114 ± 9 (10) NS	160 ± 16** (6)	143 ± 11** (7)
	(116 ± 9)	(131 ± 9)	(105 ± 16)	(115 ± 6)	(82 ± 19)	(119 ± 16)

NS: non significant, * $P \leq 0{\cdot}05$, ** $P \leq 0{\cdot}01$, *** $P \leq 0{\cdot}001$.

Twelve min after washing out the sulphydryl agents, the reversibility of the responses to the agonist was variable. Some returned to normal or 10% higher than control, others remained higher by 20% or more (Table 1).

Effects of cysteine (Table 1)
Cysteine (10–40 μg ml^{-1}) significantly increased the responses of the ileum to acetylcholine, histamine, 5-hydroxytryptamine, nicotine and bradykinin by 15–60%. The onset of action was rapid and did not increase much with time.

In most cases the responses to the agonists returned to the control values 12 min after washing out cysteine. The responses induced by PGE_2 were not significantly modified: an increase of 20–40% was observed after incubation with cysteine 10 μg ml^{-1} in 3/10 experiments and of 16–35% after incubation with 40 μg ml^{-1} in 4/10 experiments. In some experiments, an increase in spontaneous activity or a steady contraction was induced by cysteine 10 μg ml^{-1} (in 15/40 exp i.e. 37%) or 40 μg ml^{-1} (in 25/44 exp i.e. 57%). This effect was poorly reproducible.

Effects of glutathione (Table 1)
Glutathione (10–40 μg ml^{-1}) significantly increased the responses of the ileum to acetylcholine, histamine, 5-hydroxytryptamine, bradykinin and PGE_2 by 25–60%. The sensitizing effect developed rapidly and became stable after approximately 6 min of contact. In most cases, the responses to the agonists returned to the control values 12 min after washing out glutathione.

The responses induced by nicotine were not significantly modified by glutathione. An increase of 19–60% was observed after incubation with glutathione 10 μg ml^{-1} in 5/10 experiments and of 10–65% after incubation with 40 μg ml^{-1} in 4/10 experiments.

In some experiments a spasm was induced by glutathione 10 μg ml^{-1} (in 17/41 exp i.e. 41%) or 40 μg ml^{-1} (in 4/44 exp i.e. 9%). These responses were not reproducible.

Action of pharmacological antagonists
The effects of dithiothreitol and cysteine on the ileal responses to acetylcholine and histamine have been studied in the presence of various pharmacological agents acting as antagonists at different levels (n = 4 or 5 in each case). Incubation with either atropine (100 ng ml^{-1}), tetrodotoxin (100 ng ml^{-1}) or indomethacin (0·63 μg ml^{-1}) did not modify the potentiating effects of dithiothreitol or cysteine on the responses of the ileum (Table 2).

Table 2. The effects of cysteine and dithiothreitol (12 min incubation) on guinea-pig submaximal contractions induced by acetylcholine (ACh) and histamine (Hist) in the presence of various antagonists. Results are expressed in % of control submaximal contractions (mean ± s.e. mean) (n) = number of experiments. Student's *t*-test for paired data.

Antagonist in Krebs solution	Agonist	Cysteine 40 μg ml^{-1}	Dithiothreitol 40 μg ml^{-1}
Atropine 100 ng ml^{-1}	Hist	127 ± 5** (5)	135 ± 10* (4)
Tetrotodoxin 100 ng ml^{-1}	ACh	138 ± 10** (4)	123 ± 5** (4)
	Hist	129 ± 4*** (4)	132 ± 9* (4)
Indomethacin 0·63 μg ml^{-1}	ACh	123 ± 8* (4)	154 ± 8*** (5)
	Hist	125 ± 6* (4)	149 ± 4*** (4)

* $P \leq 0{\cdot}05$, ** $P \leq 0{\cdot}01$, *** $P \leq 0{\cdot}001$.

DISCUSSION

The present study has demonstrated that sulphydryl agents such as dithiothreitol (DTT), mercaptoethanol, monothioglycerol, cysteine and glutathione (these latter two being aminothiols) are able to increase non-specifically the potency of various agonists on the guinea-pig ileum. This observation was previously made for DTT and cysteine in the same preparation (Potter & Walaszek 1972; Watson & Iversen 1982) and for DTT in the guinea-pig trachea (Fleisch et al 1974), while in isolated vascular smooth muscle the effect of DTT varied according to the animal species (Fleisch et al 1973).

Glover (1979) described a selective increase of guinea-pig ileum responses to histamine by DTT but used very high concentrations of this drug (10^{-3} M) and the only agonist was acetylcholine.

The sensitizing effects appeared in our experiments at 10 μg ml^{-1} (i.e. in mol litre^{-1} respectively 6·5, 10^{-5} for dithiothreitol, 1·3, 10^{-4} for mercaptoethanol, 10^{-4} for monothioglycerol, 6·3, 10^{-5} for cysteine, 3·2, 10^{-5} for glutathione). This is a low concentration compared with that used by previous workers. In most of the cases the effects were not greater at 40 μg ml^{-1} and they were slowly or poorly reversible according to the agonist tested.

The inhibitory effect of DTT 40 μg ml^{-1} on the responses to nicotine suggests that at high concentrations DTT may exert its inhibition at the level of the ganglia located in the intramural nervous structures of the ileum. The lack of significant effect of glutathione on the ileal responses to nicotine might be explained by a balance between a sensitizing effect at the muscular level and a ganglioplegic effect.

The lack of sensitizing effect of cysteine on the ileal responses to PGE_2 remains to be explained. On the other hand, the spasm induced in some preparations by cysteine and glutathione (previously observed by Potter & Walaszek 1972 for cysteine) should be further explored.

The pharmacological analysis that we have made to elucidate the mode of action of sulphydryl agents on the guinea-pig ileum has shown that DTT and cysteine do not potentiate the ileal responses by increasing acetylcholine (or another mediator) liberation. Since these drugs exert their sensitizing effects in the presence of atropine, tetrodotoxin or indomethacin, it can be said that they act directly on the muscle cells by a mechanism in which prostaglandins are not involved.

It is well known that sulphydryl compounds are able to reduce disulphide bonds. This reduction at some vital point controlling the muscular contractility might be the best explanation for their sensitizing effects on the smooth muscle cells. However the location of these disulphide bonds is not known.

The onset of action is rapid but the effects of DTT and mercaptoethanol (and less frequently of cysteine and glutathione) increase with the time. This might suggest a rapid reduction of the disulphide bonds present on the smooth muscle cell membranes associated with a slower reduction of those located intracellularly, according to the physicochemical properties of the sulphydryl compound.

In conclusion, our results show that sulphydryl agents are able to potentiate non-specifically the responses of the guinea-pig ileum to various agonists by acting directly on the smooth muscle cells. This effect might be related to their well-described properties of reduction of disulphide bonds.

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