SPASMOLYTIC EFFECT OF POLYSORBATES (TWEENS) 80 AND 20 ISOLATED TISSUES

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Summaryy: Polysorbates (Tweens) 80 and 20 brought about 30-100% inhibition of the spasmogenic effect of histamine, acetylcholine, carbachol, angiotensin, bradykinin, 5-HT, prostaglandin F_2 alpha, prostaglandin E_1, barium chloride and potassium chloride on the isolated guinea pig ileum. Like papaverine, polysorbates blocked the contraction of the depolarized guinea pig ileum induced by calcium chloride and carbachol. Polysorbates blocked the Schultz-Dale reaction of the ileum pieces of the sensitized guinea pigs.

Polysorbates inhibited the action of carbachol and potassium chloride on the isolated rabbit jejunum, and the stimulant actions of carbachol, 5-HT, oxytocin and potassium chloride on the uterus of the oestrus-induced rat. Polysorbates also inhibited the contractions induced by adrenaline and carbachol on the isolated guinea pig seminal vesicle, but did not reduce the inhibitory effect of adrenaline and isoprenaline on the isolated rabbit jejunum. At higher doses polysorbate 80 but not 20, produced relaxation of the tissue. Polysorbates did not influence the stimulant effect of acetylcholine, carbachl and potassium chloride on the isolated frog rectus abdominis muscle.

Key words : polysorbates inhibition of Schultz Dale reaction spasmolytic effect

INTRODUCTION

Polysorbates (Tweens), the non-ionic surface active agents are extensively used for emulsifying, dispersing, wetting or dissolving many drugs (3). Unlike cationic (like cetrimide) and anionic (like teepol) agents whose respective ions are surface-active, polysorbates do not ionize in water. Oral use of polysorbate 80 is considered safe in man (11) and is recommended to promote absorption of the ingested fat and vitamin A in patients suffering from steatorrhoea (8).

Polysorbates release histamine in the dog and other species of the canine family but not in other laboratory animals and man (9, 10). In the frog *Rana tigrina*, polysorbate 80 turns the brown skin yellow (1) and induces severe hypotension and myocardial depression (7). Spermicidal and weak antihyaluronidase actions of polysorbate 80 have also been reported (18).

In pharmacodynamic studies, polysorbates are used for suspending or emulsifying drugs; in this connection Singh (15) observed that polysorbate 80 reduced the excitability of the isolated guinea pig ileum. Further work which confirmed and extended his finding is presented here.

MATERIALS AND METHODS

Polysorbate 80 known as Tween 80 or polyoxyethylene (20) sorbitan mono-oleate (sp. gr. 1.08 at 20° C, B.P.C. 1968) and polysorbate 20 known as Tween 20 or polyoxyethylene (20) sorbitan mono-laurate (sp. gr. 1.10 at 20° C B.P.C. 1968) were suitably diluted in distilled water.

Pieces of the *guinea pig ileum* (4a) and *ileum of the sensitized guinea pig* (for Schultz-Dale reaction; 13), *rabbit jejunum* (4b), *uterus of the oestrogenized rat* (13), *guinea pig seminal vesicle* (16) *and frog rectus abdominis muscle* (4c) were mounted in a 10 *ml* capacity organ bath containing Tyrode (37°C), Ringer (37°C) de Jalon (31°C), Locke (39°C) and amphibian Ringer (room temperature) solutions respectively. The bath fluids were continuously bubbled with oxygen. In some experiments, guinea pig ileum pieces were used after partial or complete depolarization (5, 6). Simple or frontal writing lever of about 1:8 magnification was used for recording the contractions on the smoked drum. In most of the experiments, separate tissue pieces were used for individual agonists. The submaximal (about 50-75% of the maximal) concentrations of the agonists were used. The individual concentrations of agonists were allowed to act for 15-60 sec till the tissues responded completely. Solution of polysorbate act for 1-3 min. Three to 5 min interval was allowed 80 or 20 was put in the bath, in 0.1 - 0.5 ml volumes, and allowed to between successive additions of the agonist; when polysorbates reduced tissue sensitivity to the agonists, sufficient time was allowed for recovery.

RESULTS

Doses mentioned below are per *ml* of the bath fluid.

Guinea pig ileum : In the concentrations which did not manifest any direct action polysorbates inhibited by about 30-100% the responses to histamine, acetylcholine, carbachol, angiotensin, bradykinin, 5-hydroxytryptamine (5-HT), prostaglandin F_2 alpha, prostaglandin E_1, barium chloride and potassium chloride (Table I). Papaverine (10 μg) which was used as a positive control reduced the response to histamine (20 *ng;* 2 experiments) carbachol (100 *ng;* 2 experiments) prostaglandin F_2 alpha (10 *ng;* 1 experiment) and prostaglandin E_1 (20 *ng;* 2 experiments) by about 50, 40, 90 and 95% respectively. The contractions of the depolarized guineapig ileum induced by calcium chloride and carbachol were inhibited by polysorbate 80 and 20 as also by papaverine (10 μg; 2 experiments).

Polysorbates blocked the antigen (horse serum)- induced contraction of the ileum of the sensitized guinea pigs (*Schultz-Dale reaction;* Table I).

Rabbit jejunum : Polysorbates inhibited the contractions induced by carbachol and potassium chloride (Table II). On the other hand polysorbate 80 (3.0 μl) and polysorbate 20 (6.0 μl) did not alter the dose-dependant relaxation induced by adrenaline (0.5 μg; 2 experiments) and isoprenaline (3 μg; 1 experiment).

In larger doses (1.0 - 3.0 μl) polysorbate 80 but not 20, inhibited the pendicular movements and produced dose-dependant relaxation (Fig. 1; 6 experiments) of the rabbit jejunum. Propranolol (Inderal, 5 μg, 2 experiments) and tolazoline (Priscol, 4 μg, 2 experiments) blocked the inhibitory action of isoprenaline (3 μg) and adrenaline (0.5 μg) but not that of polysorbate 80. Further, pentolinium (Ansolysin, 50 μg, 1 experiment) and propranolol-tolazoline mixture (1 experiment) did not influence the polysorbate 80 - induced relaxation.

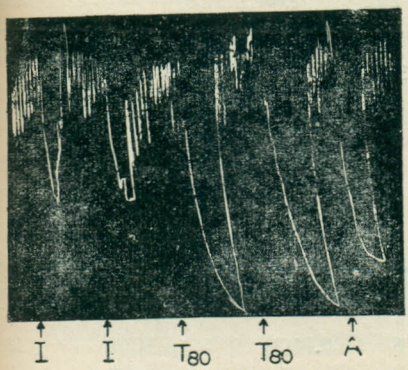

Fig. 1: Isolated rabbit jejunum. Time interval 3 min. Doses per ml bath fluid. Note that polysorbate 80 (T80, 1 *ul*) induced relaxation as did isoprenaline (I, 3 μg) and adrenaline (A, 0.05 μg).

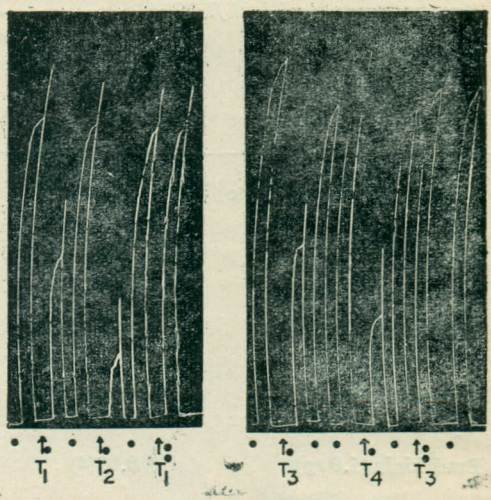

Fig.2: Isolated guinea pig illeum. Doses per ml bath fluid. Time interval 3 min. Polysorbate 80 (T_1=0.01 ul, T_2=0.03ul) and polysorbate 20 (T_3=0.01 ul, T_4=0.02ul) was added to the bath, at respective arrows, a min before carbachol (.=10ng : =20 ng.)

Rat uterus : Polysorbates 80 and 20 reduced the contractions induced by carbachol, 5-HT, oxytocin and potassium chloride (Table II).

Guinea pig seminal vesicle : Polysorbates 80 and 20 inhibited the contraction induced by adrenaline and carbachol (Table II).

Frog rectus abdominis muscle : Polysorbates 80 and 20 (upto 30 *ml* concentration) did not alter the contractions induced by acetylcholine (0.5 μg; 3 experiments), carbacol (0.5 μg; 3 experiments) and potassium chloride (1.0 *mg;* 1 experiment).

The inhibition of the agonist - induced contraction by polysorbates was dose-dependant, reversible and could be repeatedly elicited on the individual tissues; it could be overcome by increasing the dose of agonists Fig. 2. The anti-histamine effect of the polysorbates on guinea-pig ileum persisted longer (Table I) than the antagonism of the remaining agonists. The action of barium chloride, potassium chloride and calcium chloride could be inhibited by comparatively larger doses of polysorbates (Table I).

TABLE I: Inhibitory effect of polysorbates 80 and 20 on the contractile responses to different agonists of the isolated guineapig ileum.

Agonist (dose per ml bath fluid)	*Polysorbate 80*			*Polysorbate 20*		
	ul per ml bath fluid (number of experiments)	*Average (range) percent inhibition*	*Average recovery time in min*	*ul per ml bath fluid (number of experiments)*	*Average (range) percentage inhibition*	*Average recovery time in min*
Histamine acid phosphate (0.05 *ug*)	0.03(16)	45 (36—55)	36	0.03(4)	71 (60-84)	33
Acetylcholine (0.1 μg)	0.01(7)	52 (40—65)	3	0.001(3)	72 (65—82)	3
Carbachol (0.01 μg)	0.01(9)	49 (31—56)	12	0.03(5)	73 (58—85)	10
Angiotensin (5.0 *ng*)	0.03(3)	68 (53—81)	15	0.03(3)	62 (45—73)	12
Bradykinin (5.0 *ng*)	0.03(4)	50 (40—60)	6	0.01(3)	90 (80—100)	18
5-HT creatinine sulphate (0.2 μg)	0.01(3)	43 (32—50)	3	0.01(3)	62 (45—73)	3
Prostaglandin F_2 alpha (10.0μg)	0.002(3)	60 (47—80)	12	0.0016(3)	43 (35—48)	12
Prostaglandin E_1 (20.0 μg)	0.003(3)	40 (30—50)	3	0.003(3)	77 (64—90)	3
Barium chloride (10.0 μg)	0.3(4)	40 (30—55)	5	0.2(3)	95 (85—100)	12
Potassium chloride (1.0 *mg*)	0.1(5)	46 (33—60)	10	0.2(3)	92 (83—100)	22
*Calcium chloride (5.0 *mg*)	1.0(3)	50 (43—54)	15	1.0(3)	70 (64—78)	25
**Antigen (horse serum, 0.2 *ml*)	20.0(3)	100	—	5.0(3)	100	—

*Depolarized tissue used in these experiments.

**Ileum of the sentitized guineapigs were used in these experiments.

TABLE II: Effect of polysorbates 80 and 20 on the action of different agonists on some isolated tissues.

Tissues	*Agonists (dose per ml. bath fluid)*	*Polysorbate 80*			*Polysorbate 20*		
		µl per ml bath fluid (number of experiments)	*Average (range) percent inhibition*	*Average recovery time in min*	*µl per ml bath fluid (number of experiments)*	*Average (range) percent inhibition*	*Average recovery time in min.*
Rabbit jejunum*							
	Carbachol (0.05 µg)	0.03(5)	38 (30—50)	6	0.05(4)	40 (36—44)	3
	Potassium choloride (1.0 *mg*)	1.0(3)**	42 (35—53)	9	0.1(3)	38 (33—44)	6
Oestrogenized rat's uterus							
	Carbachol (0.3 µg)	0.3(3)	63 (56—73)	16	0.1(3)	42 (30—50)	12
	5-HT creatinine sulphate (10.0 *ng*)	1.4(4)	50 (40—64)	20	1.5(3)	70 (60—75)	20
	Oxytocin (0.0008 I.U.)	1.0(4)	50 (35—60)	30	0.7(4)	87 (80—93)	20
	Potassium chloride (4 mg)	2.0(3)	52 (48—60)	16	0.1(3)	46 (35—60)	12
Guinea pig seminal vesicle							
	Adrenaline (6.0 µg)	0.3(3)	70 (60—75)	35	0.1(3)	86 (75—100)	30
	Carbachol (1.0 µg)	2.0(3)	76 (70—85)	18	1.0(3)	89 (80—100)	24

*Polysorbate 80 produced dose-dependent relaxation of this tissue.
**This dose of polysorbate 80, in these experiments, did not produce relaxation.

DISCUSSION

Ideally, substances used for physical dispersion of drugs should be completely inert. However, many of them exert one or the other biological action which may interfere in the experiments. Thus, propylene glycol and sodium lauryl sulphate which are commonly used for physical dispersion of the drugs have been shown to have their own pharmacodynamic effects (14, 17). The present work shows that polysorbates are more potent inhibitors than propylene glycol on the isolated guinea pig ileum and other smooth muscles.

The earlier finding (10) that 0.01 μl of polysorbates 20 per *ml* bath fluid had no action of its own on the isolated guinea pig ileum is confirmed in this work. Blanpin (2) observed that polysorbate 80 (0.5 $\mu l/ml$ bath fluid) brought about 60% inhibition of the acetylcholine response action of the rat duodenum; this concentration and the degree of inhibition are close to those observed in the present work on the guinea pig ileum and other tissues.

It is interesting to note that polysorbates blocked the stimulant action of adrenaline on the guinea pig seminal vesicles but not its inhibitory ones on the rabbit jejunum.

Thus, the present work suggests that polysorbates inhibit only the stimulant effects of different agonists on the smooth muscle preparations.

Verges (18) has suggested that the simultaneous existence of hydrophobic and hydrophilic groups in the polysorbate molecules gives them some particular properties concerning membrance permeability. This may explain many actions of polysorbates reported in Tables I and II. However, such a mechanism would not properly explain the two findings namely- polysorbates did not block (i) the inhibitory effect of adrenaline and isoprenaline on the rabbit jejunum and (ii) the stimulant effect of acetylcholine, carbachol and potassium chloride on the frog rectus muscle.

Though polysorbate 20 (a mono-laurate ester) is a more potent inhibitor of so many agonists (Tables I and II), it failed to relax, unlike polysorbate 80 (a mono-oleate ester), the isolated rabbit jejunum. This suggests that their inhibitory action may not be related to direct muscle relaxing property.

Polysorbates, like papaverine, blocked the stimulant action of calcium chloride and carbachol on the depolarised guinea pig ileum. In the depolarized tissues, spasmogens induce contraction by directly acting on the contractile elements (5, 6). The findings in this work, therefore, suggest that polysorbates act by directly inhibiting the contractile elements of the smooth muscle.

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