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THE EFFECTS OF TIME AND INDOMETHACIN ON CONTRACTILE RESPONSES OF THE GUINEA-PIG GALL BLADDER *in vitro*

SHEILA A. DOGGRELL & GERALD W. SCOTT[1]

Department of Pharmacology and Clinical Pharmacology, School of Medicine, University of Auckland, Private Bag, Auckland, New Zealand

1 The effects of time and of indomethacin on contractile responses of the guinea-pig gall bladder were studied *in vitro*.

2 The tissues contracted to field stimulation at 5 Hz (in the absence and presence of atropine 10^{-6} M), (−)-noradrenaline (10^{-5} M), acetylcholine (10^{-5} M), and adenosine 5'-triphosphate (ATP, 10^{-4} M); the magnitude of the contractile responses increased with time.

3 (−)-Isoprenaline 10^{-5} M either relaxed (17 of 23 preparations tested) or had no effect on gall bladder strips.

4 The responses of strips of guinea-pig gall bladder to field stimulation at 5 Hz (in the absence or presence of atropine 10^{-6} M), (−)-noradrenaline (10^{-5} M), and acetylcholine (10^{-5} M) obtained 4 h 45 min after setting up the tissue were reduced following incubation with indomethacin (7.9×10^{-6} M for 1 h). The responses to (−)-isoprenaline (10^{-5} M) and to ATP (10^{-4} M) were abolished by incubation with indomethacin.

5 These results suggest that, in the guinea-pig gall bladder *in vitro*, the magnitude of the contractile responses to field stimulation at 5 Hz, (−)-noradrenaline (10^{-5} M), and acetylcholine (10^{-5} M) and the ability of the tissue to respond to (−)-isoprenaline (10^{-5} M) and ATP (10^{-4} M), may be dependent on the synthesis of a prostaglandin-like substance.

Introduction

There has been a growing interest in the neural regulation of gall bladder motility during the past decade and it is becoming apparent that there are many similarities between the responses of the gall bladder and other parts of the intestinal tract to neural stimulation. The guinea-pig gall bladder has an excitatory cholinergic innervation (Davison & Fösel, 1975) a noradrenergic innervation (Baumgarten & Lange, 1969; Davison, Al-Hassani, Crowe & Burnstock, 1978) which needs more studies to clarify its role and may also have a non-adrenergic inhibitory innervation which is reputed to be 'purinergic' (Davison *et al.*, 1978).

The present study was undertaken to ascertain the effects of field stimulation and of exogenously applied agents on the tone of the guinea-pig gall bladder, *in vitro*. In addition, as several isolated tissues of the guinea-pig have been shown to synthesize prostaglandin-like substances which can affect the tone of the preparation (ileum, Davison, Ramwell & Willis, 1972; Botting & Salzmann, 1975; trachea, Orehek, Douglas & Bouhuys, 1975; taenia coli, Sakato & Shimo, 1976), the effect of indomethacin, a prostaglandin synthesis inhibitor, on contractile responses was studied.

Methods

Adult guinea-pigs of either sex were killed by a blow to the skull and exsanguinated. The gall bladder was rapidly removed and cut into 4 approximately equal longitudinal strips. All experiments were performed in the presence of a modified Krebs solution of the following composition (mM); NaCl 116, KCl 5.4, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 22.0, D-glucose 11.2 and disodium edetate (Na_2 EDTA) 0.04, equilibrated with 5% CO_2 in O_2, at 37 C.

Gall bladder strips were mounted between 2 platinum electrodes under 1 g tension in 5 ml organ baths containing Krebs solution. This tension was maintained throughout the course of the experiment by

[1] Present address: Surgical-Medical Research Institute, The University of Alberta, Edmonton, Canada, T6G 2N8.

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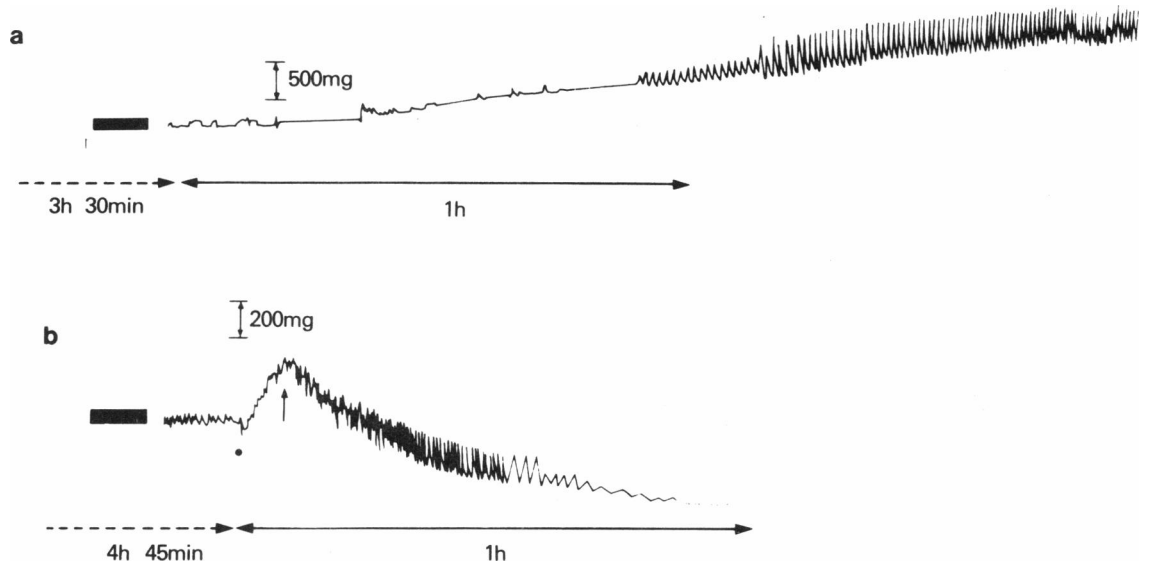

Figure 1 Guinea-pig gall bladder strip. Solid horizontal bar marks resting tension of 1 g. (a) A preparation that developed rhythmical contractions associated with a gradual increase in tone. During the period indicated the preparation was being washed by overflow and the background tension was not adjusted. (b) Another preparation showing that the rhythmical contractions and increasing tone were abolished by incubation for 1 h in the presence of indomethacin (7.9×10^{-6} M). ● End of wash.

regular adjustment. The tissues relaxed for 1 to 1 h 45 min before retaining the 1 g tension and were thus equilibrated for 1 h 45 min. Tissues were then continuously stimulated electrically (5 Hz, 1 ms duration and supramaximal voltage) or exposed to agonist for 5 min. They were then allowed to recover fully before the resting tension was adjusted to 1 g over a total period of 20 min during which the tissues were washed by overflow. The medium was not changed and the tension not adjusted for 5 min prior to further stimulation or addition of agonist. Contractile responses were recorded isometrically with force displacement transducers (Grass Model FT103.C) connected to a polygraph (Grass Model 79B).

When experiments were performed in the presence of atropine (10^{-6} M) this was present in the Krebs solution from the beginning of the equilibration period. In the studies of the effect of indomethacin on contractile responses, freshly prepared indomethacin was added to the bathing medium of the tissue to give a final concentration of 7.9×10^{-6} M for a period of 1 h, 3 h 30 min after the first response was initiated. The medium was not changed during the 1 h period in either the indomethacin-treated or time control tissue baths.

All responses were expressed as a percentage of the response obtained 3 h after the first response was initiated. This response was called the 100% or control response. The values obtained, under different conditions, were compared by Student's t test and differences were considered to be significantly different when $P < 0.05$.

The drugs used were acetylcholine chloride, (−)-adrenaline bitartrate, adenosine 5′-triphosphate (ATP), atropine sulphate, indomethacin, (−)-isoprenaline bitartrate and (−)-noradrenaline bitartrate. All compounds were obtained from Sigma Chemicals Ltd.

Results

The guinea-pig gall bladder strips were initially quiescent. In some of the preparations, development of rhythmical phasic contractions was associated with a gradual increase in tone (Figure 1a). This effect did not usually occur until 3 to 4 h after isolation. The rhythmical contractions and increasing tone were abolished by incubation in the presence of 7.9×10^{-6} M indomethacin for 1 h (Figure 1b). The gradual increase in tone in the absence and decrease in tone observed on the addition of indomethacin, necessitated the regular adjustment of background tension to maintain it at 1 g.

The tissues were contracted by field stimulation at 5 Hz and by acetylcholine 10^{-5} M. Preliminary experiments showed that the contractile responses to field stimulation at 5 Hz were reduced in magnitude

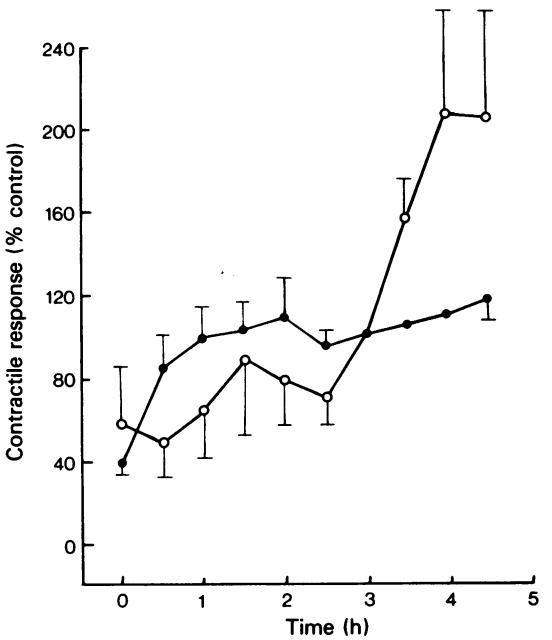

Figure 2 Effect of time on responses to field stimulation in the guinea-pig gall bladder. Responses to field stimulation at 5 Hz in Krebs solution (●) and in the presence of atropine (10^{-6} M) (○). All responses are expressed as a percentage of the response obtained 3 h after the initial response and plotted against time. Each value is the mean from 6–9 preparations; vertical lines show s.e. mean.

by approximately 80% on the addition of atropine 10^{-6} M. (−)-Noradrenaline 10^{-5} M and ATP 10^{-4} M also caused contractions. In the presence of (−)-isoprenaline 10^{-5} M, 17 of 23 gall bladder strips relaxed and the other 6 were unaffected. (−)-Adrenaline 10^{-5} M caused either a contraction, a relaxation, or a relaxation followed by a contraction.

Effect of time

The responses of the tissue to field stimulation at 5 Hz in the absence of atropine and the much smaller responses to 5 Hz obtained in the presence of atropine 10^{-6} M increased in magnitude with time (Figure 2). In the absence of atropine the responses to 5 Hz increased approximately 4 fold over a 4 h 30 min period whilst in the presence of atropine 10^{-6} M responses to 5 Hz increased 9 fold over this period (Table 1). Nevertheless at the end of the time period, the magnitude of responses to 5 Hz in the presence of atropine 10^{-6} M was still less than half that obtained in the absence of atropine (Table 1). The responses to (−)-noradrenaline 10^{-5} M, acetylcholine 10^{-5} M and to ATP 10^{-4} M also increased with time. The magnitude of the initial responses and the responses obtained 4 h 30 min later are given in Table 1.

In this series of experiments, 5 of 7 tissues relaxed in the presence of (−)-isoprenaline 10^{-5} M. In these preparations the second relaxation response was smaller than the first; the relaxant responses then increased with time (Figure 3).

Effect of indomethacin

Following incubation for 1 h in the presence of indomethacin (7.9×10^{-6} M), the responses to field stimulation (in the absence or presence of atropine (10^{-6} M): Figure 4), (−)-noradrenaline 10^{-5} M and to acetylcholine 10^{-5} M (Figure 5) were greatly reduced.

Table 1 Time study: magnitude of responses of guinea-pig gall bladder in Krebs solution

	Magnitude of response (mg) (mean ± s.e. mean)	
	Initial response[a]	*Response obtained 4 h 30 min after initial response*
Field stimulation at 5 Hz		
(a) In Krebs solution	279 ± 47 (6)	967 ± 265 (6)[1]
(b) In the presence of atropine (10^{-6} M)	43 ± 12 (8)[2]	364 ± 104 (8)[1,2]
(−)-Noradrenaline (10^{-5} M)	63 ± 16 (6)	227 ± 73 (6)[1]
Acetylcholine (10^{-5} M)	624 ± 131 (7)	894 ± 172 (7)[1]
ATP (10^{-4} M)	37 ± 14 (4)	354 ± 102 (4)[1]
(−)-Isoprenaline (10^{-5} M)	26 ± 4 (4)	43 ± 16 (14)

[1] $P < 0.05$, compared to initial response by paired t test.
[2] $P < 0.05$, compared to response in the absence of atropine by unpaired t test.
(n) = number of observations. [a]The initial response was obtained following a 1 h 45 min equilibration period.

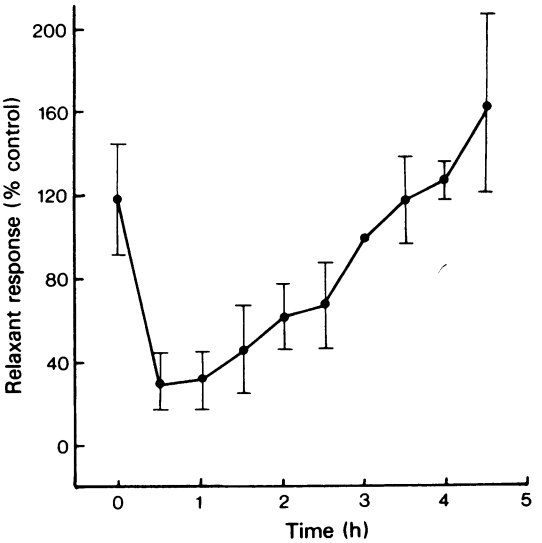

Figure 3 Effect of time on relaxant responses to (−)-isoprenaline in the guinea-pig gall bladder. Relaxant responses to (−)-isoprenaline (10^{-5} M) (●) were obtained in Krebs solution. All responses are expressed as a percentage of the response obtained 3 h after the initial response and plotted against time. Each value is the mean from 4-5 preparations; vertical lines show s.e. mean.

The contractile responses to these stimuli following indomethacin incubation were of the same order of magnitude as the initial responses. The contractile responses to ATP 10^{-4} M and the relaxant responses to (−)-isoprenaline 10^{-5} M were abolished by incubation with indomethacin (Figure 6). Following washing of the tissues, responses of increasing magnitude to all these stimuli were observed.

The time-control tissues in this series of experiments, illustrate that the magnitude of the responses to all of these stimuli (including the relaxant responses to (−)-isoprenaline 10^{-5} M) was greatly increased by 1 h incubation in Krebs solution without washing.

Discussion

The gradual increase in tone and the development of phasic contractions that occurred in our guinea-pig specimens over a period of time was similar to that reported in isolated gall bladder muscle from the cat (Persson, 1972) and man (Mack & Todd, 1968). The abolition of these changes by incubation with indomethacin suggests that the strips of gall bladder release a prostaglandin-like substance, which in turn affects the tonic and phasic activity of the muscle. Similar observations have been made with several

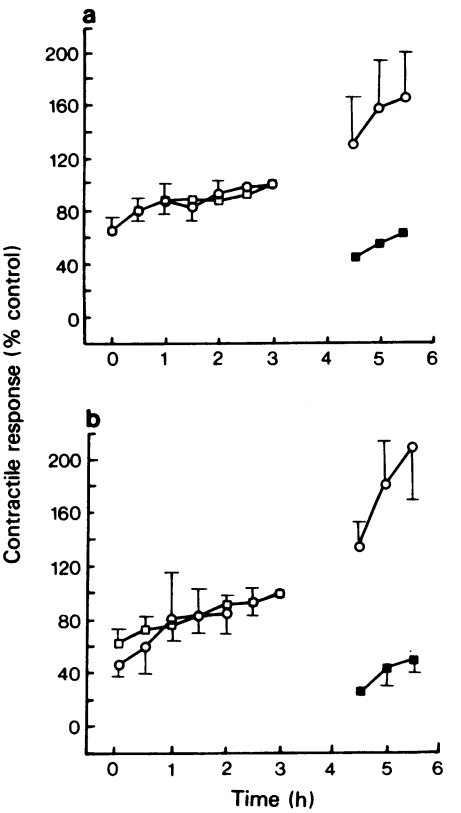

Figure 4 Effect of indomethacin on responses to field stimulation in the guinea-pig gall bladder. Responses to field stimulation at 5 Hz in Krebs solution (a) and in the presence of atropine (10^{-6} M) (b); time control responses (○) and responses before (□) and after (■) incubation in the presence of indomethacin (7.9×10^{-6} M) for 1 h. All responses are expressed as a percentage of the response obtained 3 h after the initial response and plotted against time. Each value is the mean from 4-8 preparations; vertical lines show s.e. mean.

other guinea-pig isolated tissues (ileum, Davison *et al.*, 1972; Botting & Salzmann, 1975; trachea, Orehek *et al.*, 1975; taenia coli, Sakato & Shimo, 1976), rabbit jejunum (Ferreira, Herman & Vane, 1972), rat stomach strip and chick rectum (Eckenfels & Vane, 1972).

Although a noradrenergic innervation to the guinea-pig gall bladder has been clearly demonstrated (Baumgarten & Lange, 1969; Davison *et al.*, 1978), there have been few studies of the effects of exogenously applied catecholamines on this tissue. Persson (1972) has demonstrated that strips of cat gall bladder are relaxed by (−)-noradrenaline, (−)-adrenaline, and by (±)-isoprenaline and that these relaxations increased in magnitude with time. Furthermore, following propranolol pretreatment the tissues

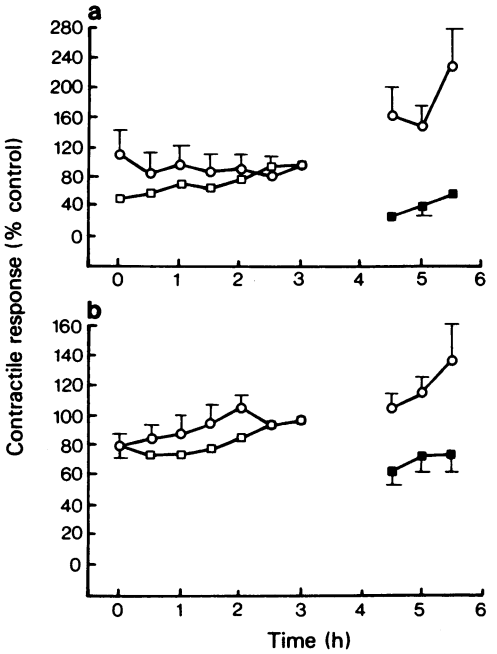

Figure 5 Effect of indomethacin on responses to (−)-noradrenaline and acetylcholine in the guinea-pig gall bladder. Responses to (−)-noradrenaline (10^{-5} M) (a) and acetylcholine (10^{-5} M) (b); time control responses (○) and responses before (□) and after (■) incubation in the presence of indomethacin (7.9×10^{-6} M) for 1 h. All responses are expressed as a percentage of the response obtained 3 h after the initial response and plotted against time. Each value is the mean from 4-8 preparations; vertical lines show s.e. mean.

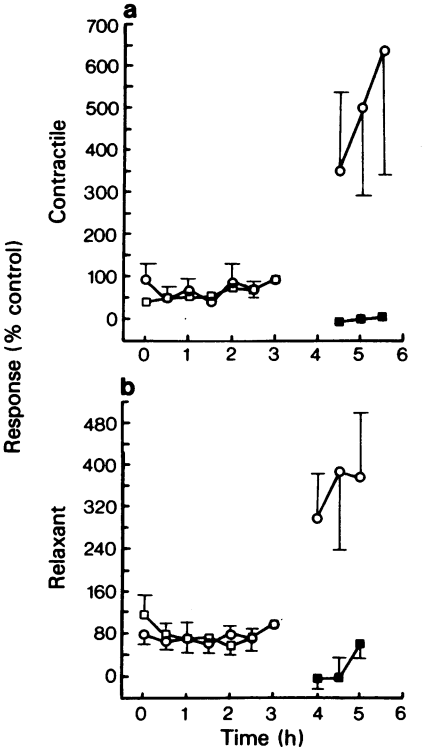

Figure 6 Effect of indomethacin on responses to ATP and (−)-isoprenaline in the guinea-pig gall bladder. Contractile responses to (a) ATP (10^{-4} M) and relaxant responses to (b) (−)-isoprenaline (10^{-5} M); time control responses (○) and responses before (□) and after (■) incubation in the presence of indomethacin (7.9×10^{-6} M) for 1 h. All responses were expressed as a percentage of the responses obtained 3 h after the initial response and plotted against time. Each value is the mean from 5-7 preparations; vertical lines show s.e. mean.

contracted in the presence of noradrenaline and adrenaline and these responses were abolished by phenoxybenzamine. Thus Persson (1972) suggested that the cat gall bladder contained both β-adrenoceptors, which mediate relaxations and α-adrenoceptors which, when stimulated, may give rise to contractions. In the present study, (−)-noradrenaline consistently induced contractions of the guinea-pig gall bladder whereas (−)-isoprenaline usually produced relaxations and never caused contractions.

The responses to field stimulation in strips of guinea-pig gall bladder were abolished by tetrodotoxin 3×10^{-6} M (unpublished observation) and greatly reduced by atropine 10^{-6} M. This illustrates the excitatory cholinergic innervation to the guinea-pig gall bladder as previously demonstrated by Davison & Fösel (1975). The nature of the residual excitatory innervation has not been determined.

The responses to field stimulation (in the absence and presence of atropine) and to exogenously applied (−)-noradrenaline and acetylcholine increased with time, this increase being very marked if the bathing medium was unchanged for an hour. The responses to field stimulation, (−)-noradrenaline and to acetylcholine were reduced by indomethacin at a concentration reported to inhibit prostaglandin synthesis (Vane, 1971). Moreover, the responses to field stimulation, (−)-noradrenaline and acetylcholine following indomethacin incubation were of a similar magnitude to the initial responses to these stimuli. This suggests that, in the guinea-pig gall bladder, the magnitude of the contractile responses to field stimulation, (−)-noradrenaline and acetylcholine may increase due to the synthesis of a prostaglandin-like substance.

It has been suggested that prostaglandins may facilitate cholinergic transmission by a presynaptic action. Thus prostaglandins have been shown to facilitate cholinergic transmission without altering re-

sponses to exogenously administered acetylcholine in the isolated ileum and trachea of the guinea-pig (Hall, O'Neil & Sheehan, 1975). Furthermore acetylsalicyclic acid, a prostaglandin synthesis inhibitor, reduces the twitch response to transmural stimulation and the output of acetylcholine in the guinea-pig ileum without altering the responses to exogenously administered acetylcholine (Hall *et al.*, 1975). However, it seems unlikely that a presynaptic action of prostaglandins is solely responsible for the effects seen in the present study, as indomethacin also reduced the responses to exogenously applied acetylcholine.

The relaxant responses to (−)-isoprenaline and the contractile responses to ATP were abolished by indomethacin. This suggests that the ability of the guinea-pig gall bladder to respond to (−)-isoprenaline and ATP may be dependent on the synthesis of a prostaglandin derivative. The ability of ATP to induce prostaglandin-mediated contractile responses of this tissue has previously been demonstrated by Davison *et al.* (1978).

Davison *et al.* (1978) have recently reported that the isolated intact gall bladder of the guinea-pig contracted during transmural electrical stimulation but relaxed during stimulation in the presence of atropine. These relaxations were not abolished by the addition of adrenoceptor antagonists or by an adrenergic neurone blocker but were abolished by tetrodotoxin or lignocaine and this led Davison *et al.* (1978) to propose that the relaxations were due to stimulation of non-adrenergic nerves. Furthermore, because the nerve-mediated relaxations were mimicked by ATP and adenosine, blocked by quinidine, and potentiated by dipyridamole, they proposed that these inhibitory neurones were purinergic. This was supported by their observation that nerve cell bodies and fibres exhibited fluorescence after incubation of gall bladder specimens in quinacrine.

In our study, in which the strips of gall bladder were maintained at a resting tension of 1 g, it was possible to induce and record relaxations, for example to (−)-isoprenaline. However, field stimulation, in the absence or presence of atropine and exogenously applied ATP, before or after inhibition of prostaglandin synthesis, always caused contractions only. Thus we have no evidence of an inhibitory, possibly purinergic, innervation of guinea-pig gall bladder.

Finally, in summary, we propose that prostaglandin-like substances are produced by the guinea-pig gall bladder, at least when strips are maintained in organ baths and that under these conditions they affect the resting tone and responses to neural and exogenously applied stimuli. Experiments must therefore be carefully controlled to avoid unwanted time-dependent or modulating effects of prostaglandins. The extent to which prostaglandins are released or affect gall bladder motility *in vivo* is unknown and requires investigation.

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References

BAUMGARTEN, H.G. & LANGE, W. (1969). Extrinsic adrenergic innervation of the extrahepatic biliary duct system in guinea-pigs, cats and rhesus monkeys. *Z. Zellforsch*, **100**, 606–615.

BOTTING, J.H. & SALZMANN, R. (1974). The effect of indomethacin on the release of prostaglandin E_2 and acetylcholine from guinea-pig isolated ileum at rest and during field stimulation. *Br. J. Pharmac.*, **50**, 119–124.

DAVISON, J.S., AL-HASSANI, M., CROWE, E. & BURNSTOCK, G. (1978). The non-adrenergic inhibitory innervation of the guinea-pig gall bladder. *Pflügers Arch.* **377**, 43–49.

DAVISON, J.S. & FÖSEL, S. (1975). Interactions between vagus nerve stimulation and pentagastrin or secretin on the guinea-pig gall bladder. *Digestion*, **13**, 251–254.

DAVISON, P., RAMWELL, P.W. & WILLIS, A.L. (1972). Inhibition of intestinal tone and prostaglandin synthesis by 5, 8, 11, 14 tetraynoic acid. *Br. J. Pharmac.*, **46**, 547–548P.

ECKENFELS, A. & VANE, J.R. (1972). Prostaglandins, oxygen tension and smooth muscle tone. *Br. J. Pharmac.*, **45**, 451–462.

FERREIRA, S.H., HERMAN, A. & VANE, J.E. (1972). Prostaglandin generation maintains the smooth muscle tone of the rabbit isolated jejunum. *Br. J. Pharmac.*, **44**, 328–330P.

HALL, W.J., O'NEIL, P. & SHEEHAN, J.D. (1975). The role of prostaglandins in cholinergic neurotransmission in the guinea-pig. *Eur. J. Pharmac.*, **34**, 39–47.

MACK, A.J. & TODD, K.J. (1968). A study of human gall bladder *in vitro*. *Gut*, **9**, 546–549.

OREHEK, J., DOUGLAS, J.S. & BOUHUYS, A. (1975). Contractile responses of the guinea-pig trachea *in vitro*: Modification by prostaglandin synthesis inhibiting drugs. *J. Pharmac. exp. Ther.*, **194**, 554–564.

PERSSON, C.G.A. (1972). Adrenoceptors in the gall bladder. *Acta pharmac. tox.*, **31**, 177–185.

SAKATO, M. & SHIMO, Y. (1976). Possible role of prostaglandin E_1 on adrenergic neurotransmission in the guinea-pig taenia coli. *Eur. J. Pharmac.*, **40**, 209–214.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action of aspirin-like drugs. *Nature, New Biol.*, **231**, 232–235.

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