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19. ABSTRACT (Continue on reverse if necessary and identify by block number) Oligomeric mixtures of prostaglandin B ₁ (O-PGB) relaxes airways of guinea pig when studied in vitro. Bronchi with the epithelium preserved or removed were exposed to contractile agent for 20 minutes either in presence or in absence of indomethacin 5x10 ⁻⁶ M. O-PGB was added at 10 ⁻⁶ and 10 ⁻⁵ M when present, and the results were defined in terms of percent of maximum relaxation induced by papaverine 10 ⁻³ M. Substance P (SP) 3x10 ⁻⁸ M, leukotriene D4 (LTD4) 1x10 ⁻⁹ M and carbachol 1x10 ⁻⁸ M induced similar contractions. The degree of relaxation induced by O-PGB was dependent upon the contractil against (36% of papaverine when the bronchi were precontracted with SP, 25%-against LTD4 and only 15% against carbachol) suggesting that the mechanism is specific. The relaxation was not altered by removing the epithelial cells or by the addition of indomethacin, therefore, the effect of O-PGB seems to occur independently of cyclooxygenase-or (over)			
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or epithelium- derived substances. We also analysed the effect of O-PGB on "acute" and "chronic" preparations in regard to immediate hypersensitivet phenomenon. Animals were actively sensitized to ovalbumin (OVA) 3 weeks prior to testing to OVA in vitro. In "acute" experiments airways were exposed to OVA in vitro with or without the presence of O-PGB at $10^{-5}M$, followed by a maximal contraction to $BaCl_2$, $3 \times 10^{-2}M$. No difference was found. "Chronic" experiments with sensitized animals were carried out in airway segments incubated for 18 or 24 hours in RPMI 1640 medium with or without O-PGB. The contractil response were identical after 18 hours. We found that bronchi incubated for 24 hours with O-PGB showed a significant increase in OVA-induced contractions. Indomethacin $5 \times 10^{-6}M$ added to the tissue bath enhanced the control dcse-response curve but failed to further increase the responses of O-PGB-treated tissues. These experiments seem to show that O-PGB has two distinct effects: 1) acutely it relaxes precontract airways in a agonist-dependent manner and 2) increases the hypersensitivity responses of bronchi isolated from sensitized animals, when incubated 24 hours.

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Final Report on Contract N00014-86-K-0490

Principal Investigator: James A. Will, D.V.M., Ph.D.

Contractor: Board of Regents. University of Wisconsin-Madison

Contract Title: Pharmacologic Studies on the *In Vitro* Bronchodilating Vasoactive Actions of Oligo-PGB

Start Date: 1 August, 1986

Research Objective: To determine if oligo-PGB has effects on the smooth muscle of airway and vasculature of guinea pigs and humans studied in vitro.

We have determined that oligo-prostaglandin B (oligo-PGB) relaxes airways of guinea pigs when studied in vitro. In this study we used both high molecular weight mixture (HMW) and oligo-PGB forms of the compound. We also investigated whether or not the relaxation is dependent upon the concentration of oligo-PGB, the contractile substance used to enhance tone, the integrity of epithelial cells layer and the release of cyclooxygenase metabolites. We further analysed the effect of oligo-PGB on "acute" (protocol IV) and "chronic" (protocol V) preparations in regard to the immediate hypersensitivity phenomenon. In the chronic preparation, the tissue was incubated in the presence of oligo-PGB for 18 or 24 hours.

In protocol I, we either tested HMW and oligo-PGB in the absence of a contractile agent or after exposing the tissue to carbachol (Cch) for 20 minutes. The studies were further differentiated by doing the experiments either in the presence or absence of indomethacin, a prostaglandin synthesis inhibitor at 5 times 10^{-6} M and the results are defined in terms of percent of maximum relaxation induced by papaverine added at 10^{-3} M.

We compared the activity of HMW with that of oligo-PGB. HMW relaxed to a lesser degree than oligo-PGB at 10^{-6} M, but caused slight contractions at 10^{-5} M when no agent was used to precontract the airway (bronchi). This protocol relies on the inherent tone of the tissues. When airways were precontracted with carbachol (Cch) at 3×10^{-8} M, the results were similar.

We next attempted to determine the optimal conditions for the relaxation. From this point we did all experiments with the trimer, oligo-PGB. When

tissues were precontracted with Cch, there appeared to be a trend toward decreased relaxation with a decrease in extracellular Ca^{++} from 2.5 to 0.5 mM. The level of contraction with Cch was unaltered in a low Ca^{++} substrate.

In protocol II, tissues with the epithelium preserved or removed were exposed to the contractile agent for 20 minutes either in presence or in the absence of indomethacin ($5 \times 10^{-6}\text{M}$). oligo-PGB was added at 10^{-6} (group A) and 10^{-5}M (group B). When bronchi were precontracted with substance P (SP), or leukotriene D_4 (LTD_4) the magnitude of contraction was similar to that of Cch. SP-precontracted tissues relaxed more than LTD_4 or Cch (figure 1 and table 1). In SP and LTD_4 -precontracted tissues there was a tendency for a dose-dependent relaxation (figure 1 and table 1). There were no differences between the relaxation responses with Cch precontracted tissues at either 10^{-5} or 10^{-6}M with the exception of the group with intact epithelial cells and in the presence of indomethacin which showed a greater degree of relaxation at 10^{-5}M than at 10^{-6}M of oligo-PGB. We suggest that the complete dose-response curve to oligo-PGB will be the best protocol to study this relationship.

To exclude the possibility that the oligo-PGB relaxation could be due to a release of an inhibitory prostaglandin, we blocked production of prostaglandins by pretreating the tissues with indomethacin. Pretreatment did not affect the amount of tone induced by the different contractile agonists nor the magnitude of relaxation induced by oligo-PGB.

We also considered the possibility that bronchial epithelial cells could interact with the activity of oligo-PGB. We tested this hypothesis by mechanically removing the epithelial cells of the bronchi. This procedure did not alter the activity of oligo-PGB, with the exception of the tissues precontracted with Cch in the presence of indomethacin where oligo-PGB at 10^{-6}M elicited twice as much relaxation in tissues with the epithelial cells removed than with the matched control (Figure 1 and table 1). We are unable to explain these differences. It appears that an epithelium-derived factor does not participate in the relaxation induced by oligo-PGB.

In protocol III, dose response curves to SP and LTD_4 were obtained in the presence or absence of oligo-PGB added 20 minutes before the compound in study. oligo-PGB at 10^{-5} failed to exhibit any antagonism of SP (figure 2) or LTD_4 (figure 3) contractile responses.

Protocols IV and V, were designed to determine if oligo-PGB plays a role in the immediate hypersensitivity response. Animals were actively

sensitized to ovalbumin (OVA) 3 weeks prior to testing. In "acute" experiments (protocol IV), airways were exposed to OVA *in vitro* with or without the presence of oligo-PGB at 10^{-5}M , followed by a maximal contraction to BaCl_2 ($3 \times 10^{-2}\text{M}$). No difference was found (figure 4).

"Chronic" experiments (Protocol V) with sensitized animals were carried out in airway segments incubated for: 18 (overnight) or 24 hours in RPMI 1640 media (Sigma Chemical, Co.) with or without oligo-PGB and then dose response curves to OVA were run in the presence or absence of oligo-PGB and compared to the maximal contraction by BaCl_2 . The contractile responses to OVA were identical after 18 hours of incubation with or without oligo-PGB (figure 5). In contrast, we found that in bronchi incubated for 24 hours with oligo-PGB, the contractions to OVA increased (figure 6). While 18 hours had no effect, and the fact that 24 hours showed a significant increase in contraction would imply that there is a critical time of exposure to oligo-PGB in order to cause these differences. This does not appear to be reasonable and we need to increase the number of experiments with 18 hours of incubation. In our previous progress report, only the 18 hours incubation information was included. Indomethacin ($5 \times 10^{-6}\text{M}$) added to the bath of tissues incubated for 24 hours enhanced the responses of control but failed to further increase the responses of O-PGB-treated tissues (figure 7).

We also examined the dose response curve to OVA in the presence of pyrilamine (histamine H_1 -receptor antagonist) alone or in combination with indomethacin. Pyrilamine reduced the response of tissues incubated for 24 hours to OVA, regardless of the presence or absence of oligo-PGB in the incubation medium (figure 8). The combination of indomethacin and pyrilamine abolished the differences between control and O-PGB-treated tissues (figure 9).

These experiments seem to show that oligo-PGB has two distinct effects:

- 1) Acutely it relaxes precontracted airways in an agonist-dependent manner.
- 2) oligo-PGB increases the hypersensitivity responses of bronchi isolated from sensitized animals, when incubated for 24 hours.

The conclusions of these experiments are:

- 1) oligo-PGB relaxes airways.
- 2) Relaxation by oligo-PGB is dependent upon the contractile agonist; suggesting that the mechanism is specific.
- 3) Relaxation by oligo-PGB occurs independently of cyclooxygenase- or epithelium-derived substances.
- 4) Treatment with oligo-PGB may alter the immediate hypersensitivity phenomenon after 24 hours implying that the alteration requires time to occur. Although the mechanism by which oligo-PGB increases the responses to OVA is not known, the experiments with indomethacin indicate that oligo-PGB might have some effect on arachidonic acid metabolism.

Legend Figure 1.

Relaxation induced by oligo-PGB in guinea pig isolated bronchi expressed as percent of the maximum induced by papaverine ($1 \times 10^{-3} \text{M}$). Tissues were contracted with substance P ($3 \times 10^{-8} \text{M}$), LTD_4 ($1 \times 10^{-9} \text{M}$) or carbachol ($1 \times 10^{-8} \text{M}$). The bronchi had the epithelium preserved or mechanically removed. Indomethacin ($5 \times 10^{-6} \text{M}$) was presented for 2 hours, where indicated. Letters of each column corresponded to "t" test of table 1. N=8 for all groups, except for: group K (N=9), S (N=6), U (N=6) and Z (N=10).

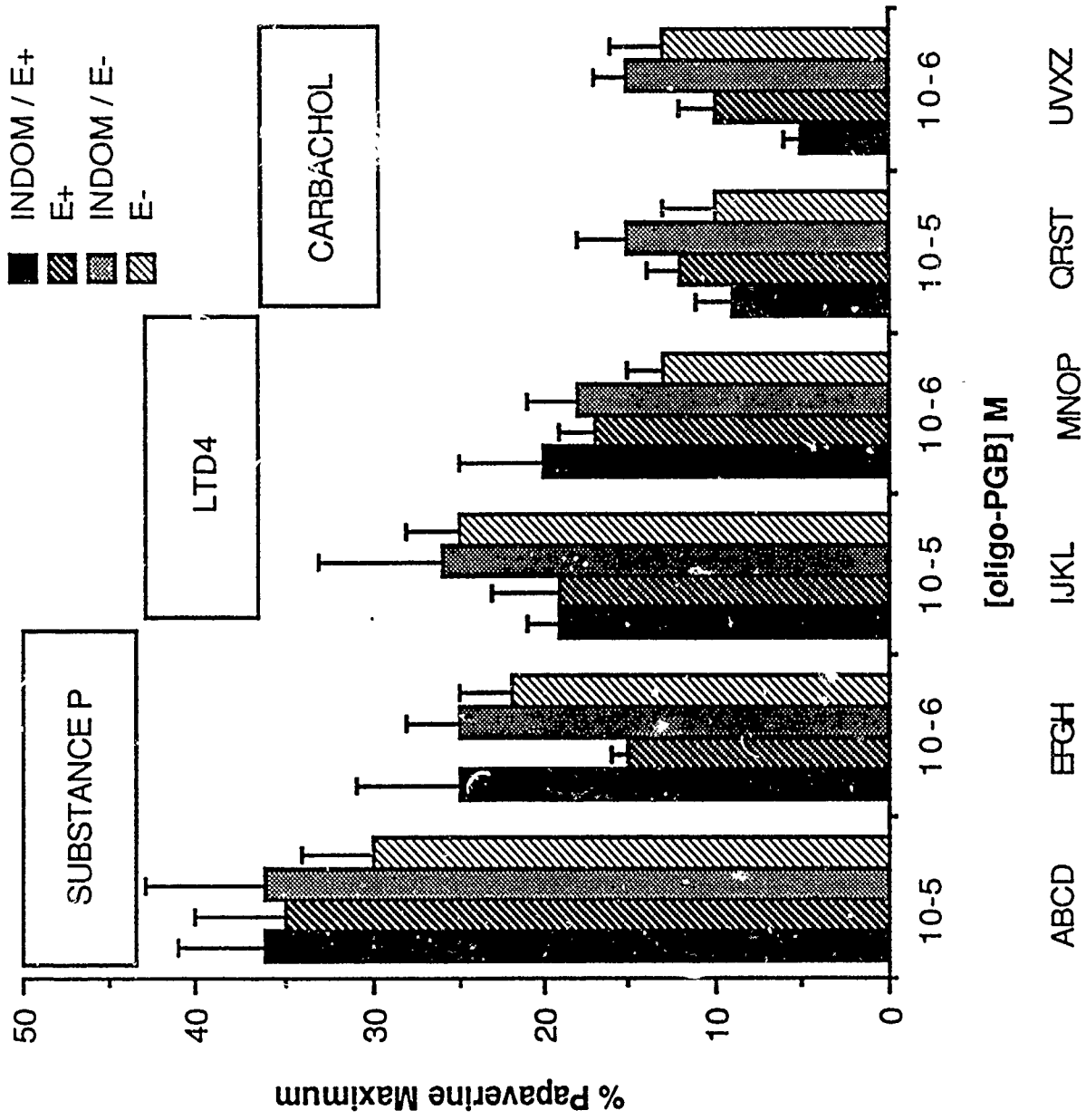


FIG.1

ANALYSIS	[oligo-PGB]	TREATMENT	COLUMN FIG.1	D.F.	t VALUE
SP X LTD4	5	E+ / INDO	A / I	14	3.19*
SP X LTD4	5	E+	B / J	14	2.50*
SP X LTD4	5	E- / INDO	C / K	14	1.01
SP X LTD4	5	E-	D / L	14	0.87
SPXCCH	5	E+ / INDO	A / Q	14	4.10*
SPXCCH	5	E+	B / R	14	4.13*
SPXCCH	5	E- / INDO	C / S	15	2.83*
SPXCCH	5	E-	D / T	14	4.11*
SP X LTD4	6	E+ / INDO	E / M	14	0.72
SP X LTD4	6	E+	F / N	14	0.05
SP X LTD4	6	E-	G / O	12	1.53
SP X LTD4	6	E- / INDO	H / P	14	2.17
SPXCCH	6	E+ / INDO	E / U	14	3.40*
SPXCCH	6	E+	F / V	14	2.83*
SPXCCH	6	E- / INDO	G / X	14	2.30*
SPXCCH	6	E-	H / Z	16	1.84
LTD4 X CCH	5	E+ / INDO	I / Q	14	3.39*
LTD4 X CCH	5	E+	J / R	14	1.3
LTD4 X CCH	5	E- / INDO	K / S	15	1.09
LTD4 X CCH	5	E-	L / T	14	3.79*
LTD4 X CCH	6	E+ / INDO	M / U	14	2.96*
LTD4 X CCH	6	E+	N / V	14	2.25*
LTD4 X CCH	6	E- / INDO	O / X	12	0.59
LTD4 X CCH	6	E-	P / Z	16	0.11
SP					
+INDO / -INDO	5	E+	A / B	14	0.15
+INDO / -INDO	5	E-	C / D	14	0.74
+INDO / -INDO	6	E+	E / F	14	1.41
+INDO / -INDO	6	E-	D / H	14	0.56
SP					
E+ / E-	5	INDO	A / C	14	0.01
E+ / E-	5		B / D	14	0.72
E+ / E-	6	INDO	E / G	14	0.12
E+ / E-	6		F / H	14	1.25
SP					
10-5 / 10-6		E+ / INDO	A / E	14	1.38
10-5 / 10-6		E+	B / F	14	3.58*
10-5 / 10-6		E- / INDO	C / G	14	1.56
10-5 / 10-6		E-	D / H	14	1.48

TABLE 1. STATISTICAL ANALYSIS OF THE DATA FROM FIGURE 1 * indicates $p < 0.05$

LTD4					
+INDO / -INDO	5	E+	I / J	14	0.04
+INDO / -INDO	5	E-	K / L	14	0.11
+INDO / -INDO	6	E+	M / N	14	0.53
+INDO / -INDO	6	E-	O / P	12	1.37
LTD4					
E+ / E-	5	INDO	I / K	14	1.03
E+ / E-	5		J / L	14	1.2
E+ / E-	6	INDO	M / O	12	0.37
E+ / E-	6		N / P	14	1.43
LTD4					
10-5 / 10-6		E+ / INDO	I / M	14	1.03
10-5 / 10-6		E+	J / N	14	0.35
10-5 / 10-6		E- / INDO	K / O	12	1.03
10-5 / 10-6		E-	L / P	14	3.11*
CCH					
+INDO / -INDO	5	E+	Q / R	14	1.21
+INDO / -INDO	5	E-	S / T	15	1.26
+INDO / -INDO	6	E+	U / V	14	1.95
+INDO / -INDO	6	E-	X / Z	16	0.66
CCH					
E+ / E-	5	INDO	Q / S	15	1.73
E+ / E-	5		R / T	14	0.67
E+ / E-	6	INDO	U / X	14	4.17
E+ / E-	6		V / Z	16	0.59
CCH					
10-5 / 10-6		E+ / INDO	Q / U	14	1.43
10-5 / 10-6		E+	R / V	14	0.67
10-5 / 10-6		E- / INDO	S / X	15	0.73
10-5 / 10-6		E-	T / Z	16	0.57

TABLE 1. STATISTICAL ANALYSIS OF THE DATA FROM FIGURE 1. * indicates $p < 0.05$

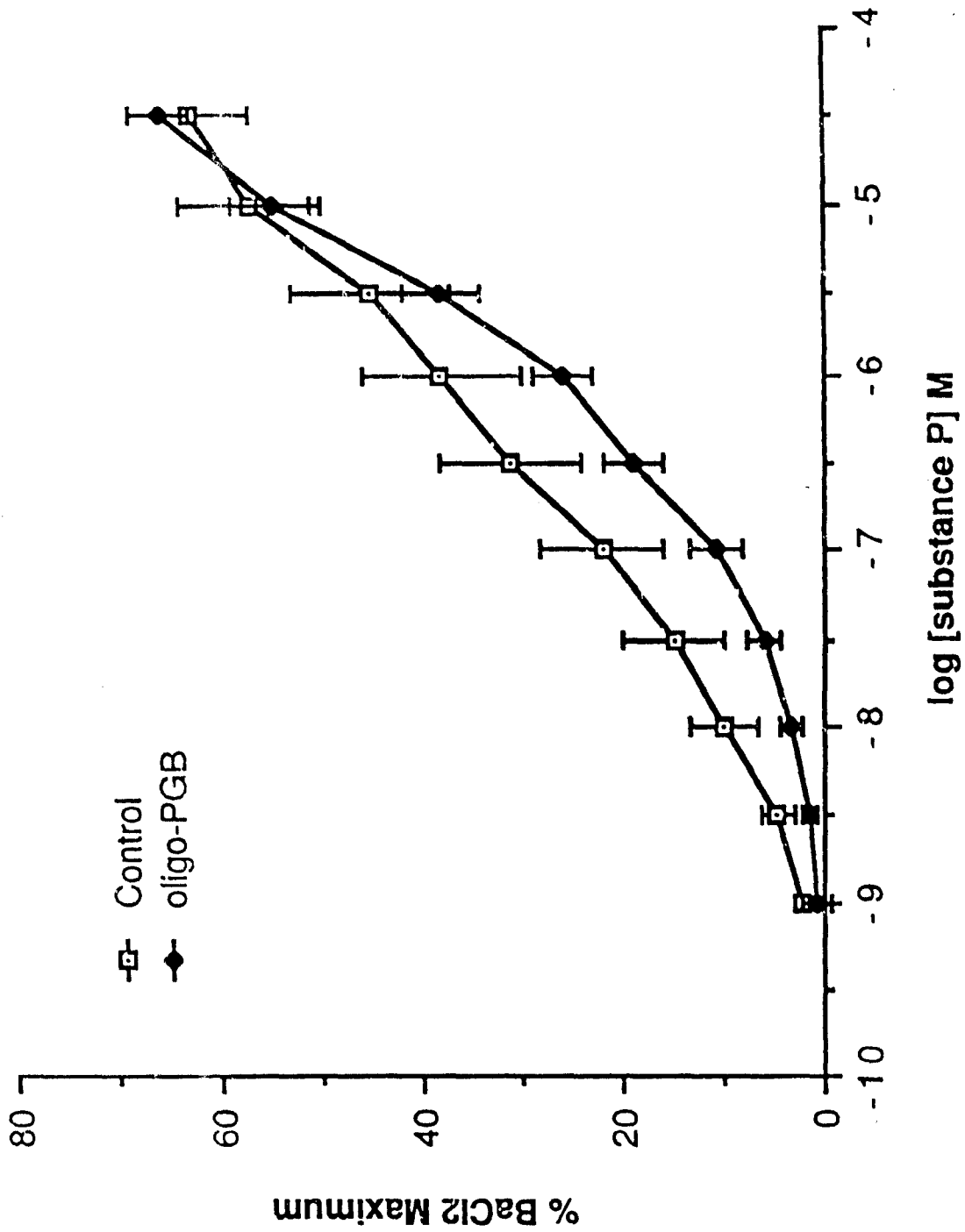


FIG.2 Dose response curve to substance P in presence (N=8) or in absence of oligo-PGB.

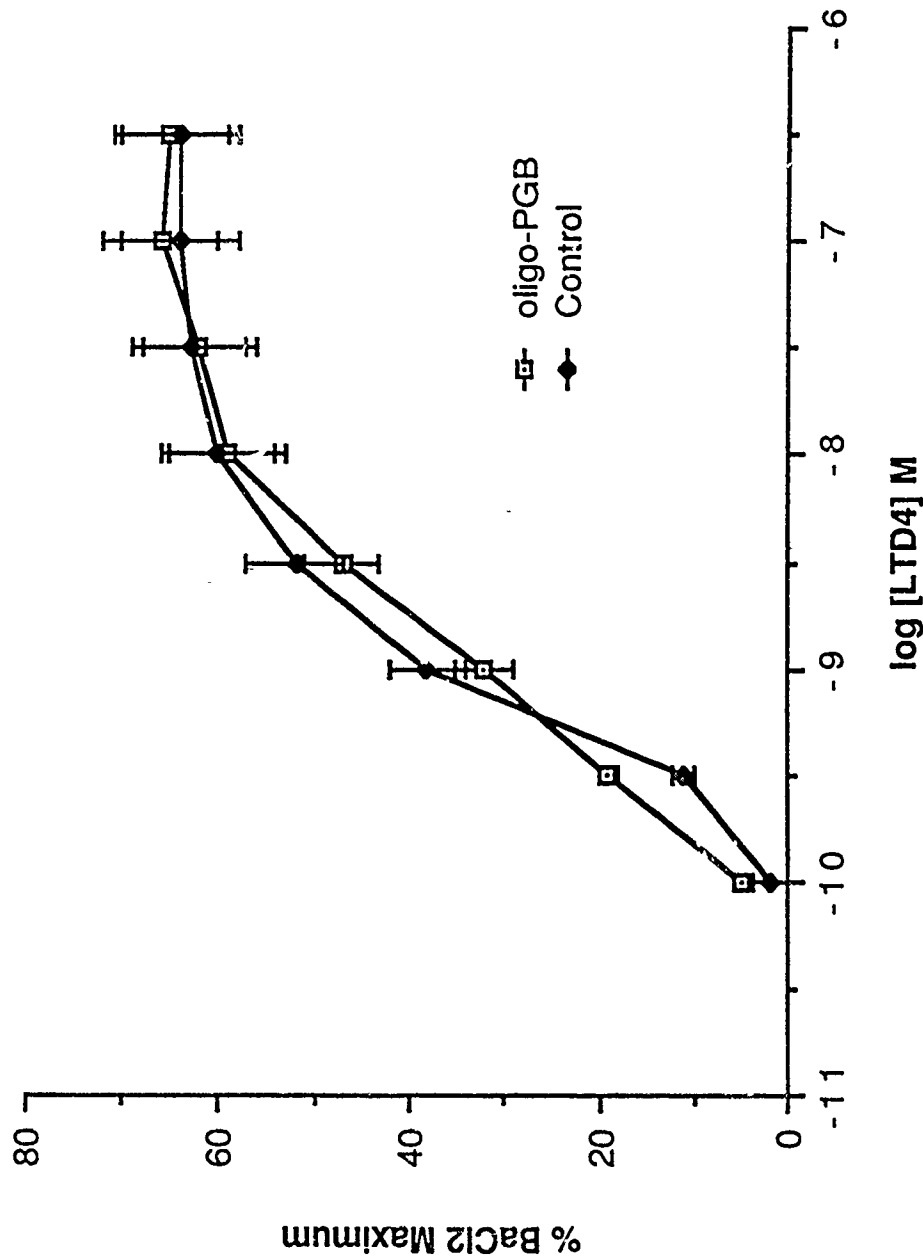


FIG.3 Dose response curve to LTD4 in the presence (N=4) or absence (N=4) of oligo-PGB.

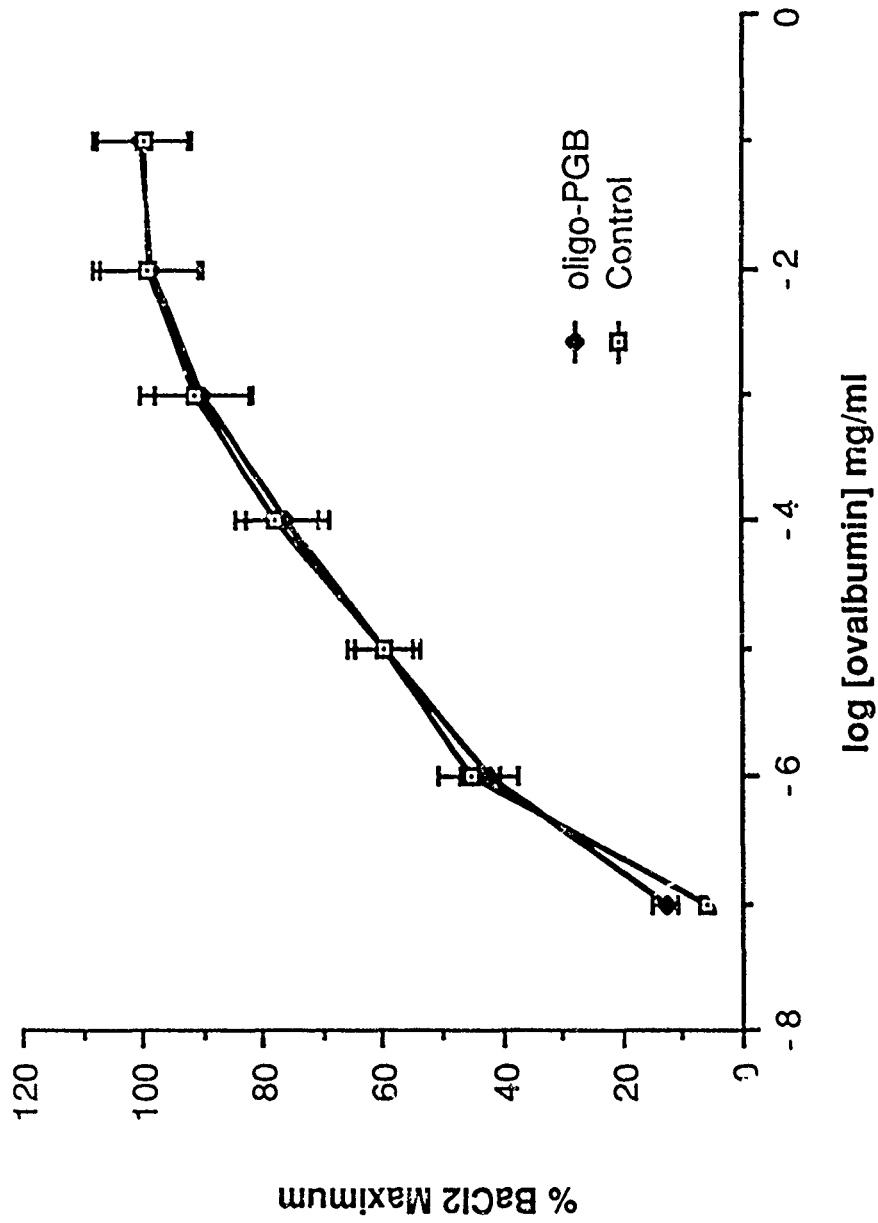


FIG.4 Dose response curve to ovalbumin in left bronchi isolated from sensitized guinea pigs, in the presence of indomethacin 5×10^{-6} M. Oligo-PGB (10^{-5} M) was added 20 minutes before. N=6 in both groups.

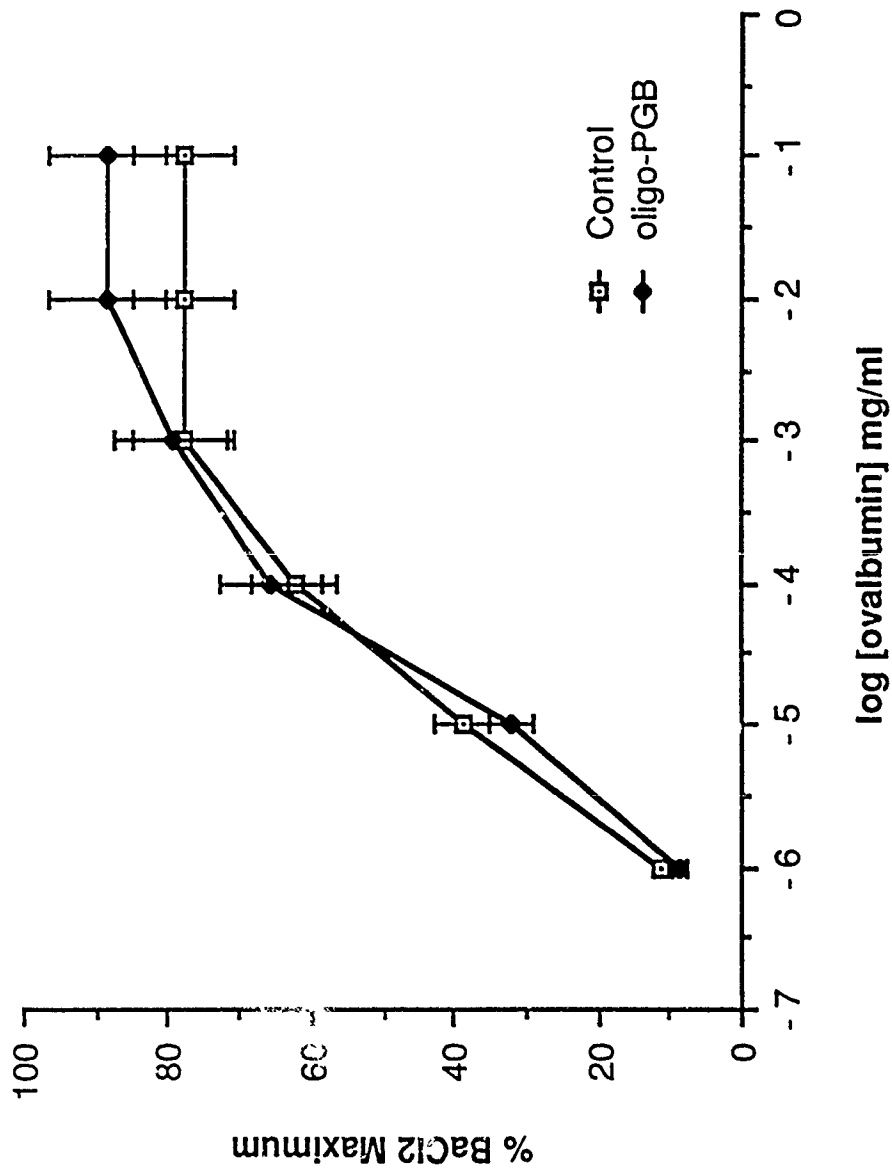


FIG.5 Dose response curve to ovalbumin in left bronchi isolated from sensitized guinea pigs and incubated for 18 hours in RPMI 1640 media with or without oligo-PGB (10^{-5} M). N=4 in both groups.

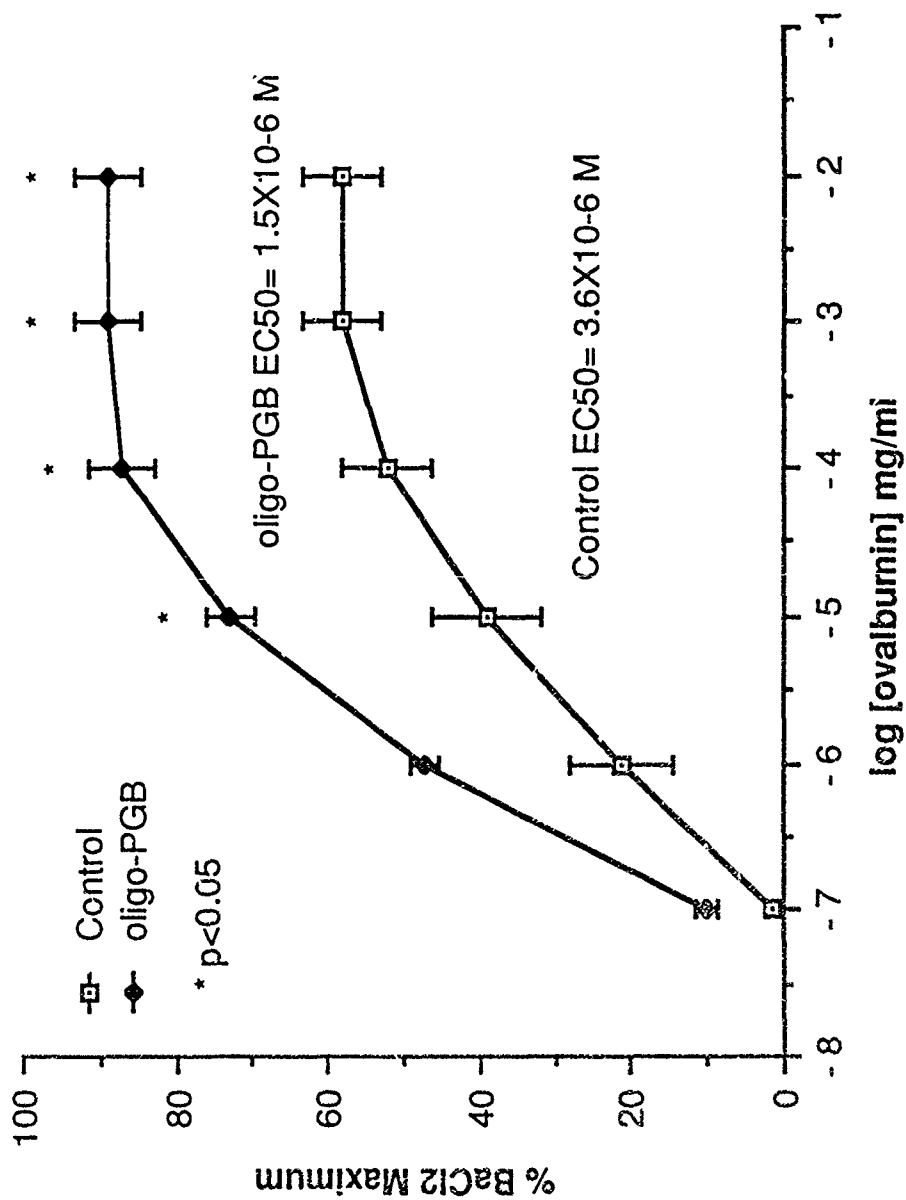


FIG.6 Dose response to ovalbumin in left bronchi isolated from sensitized guinea pigs and incubated for 24 hours in RPMI 1640 media with or without oligo-PGB (10⁻⁵ M). N=8 in both groups.

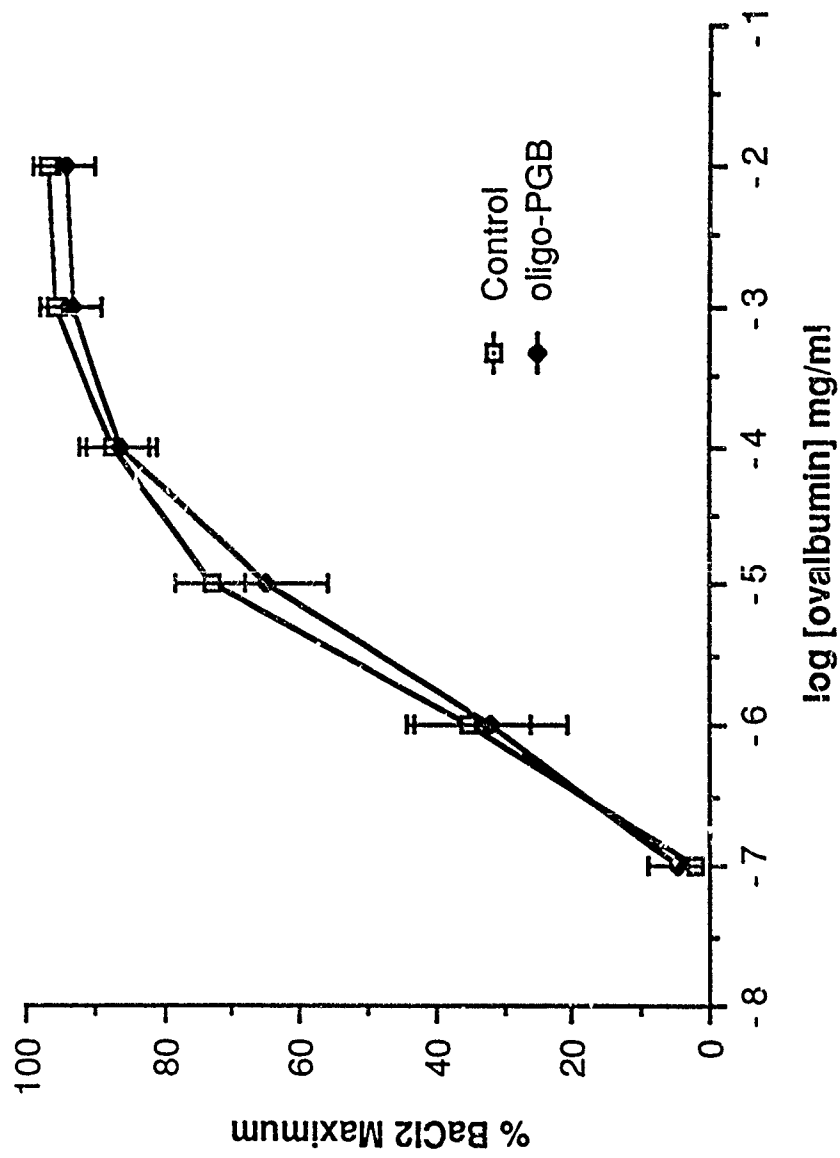


FIG.7 Dose response curve (DRC) to ovalbumin in left bronchi isolated from sensitized guinea pigs and incubated for 24 hours in RPMI 1640 media with or without oligo-PGB (10^{-5} M). Indomethacin 5×10^{-6} M was added 2 hours before the DRC. N=8 in both groups.

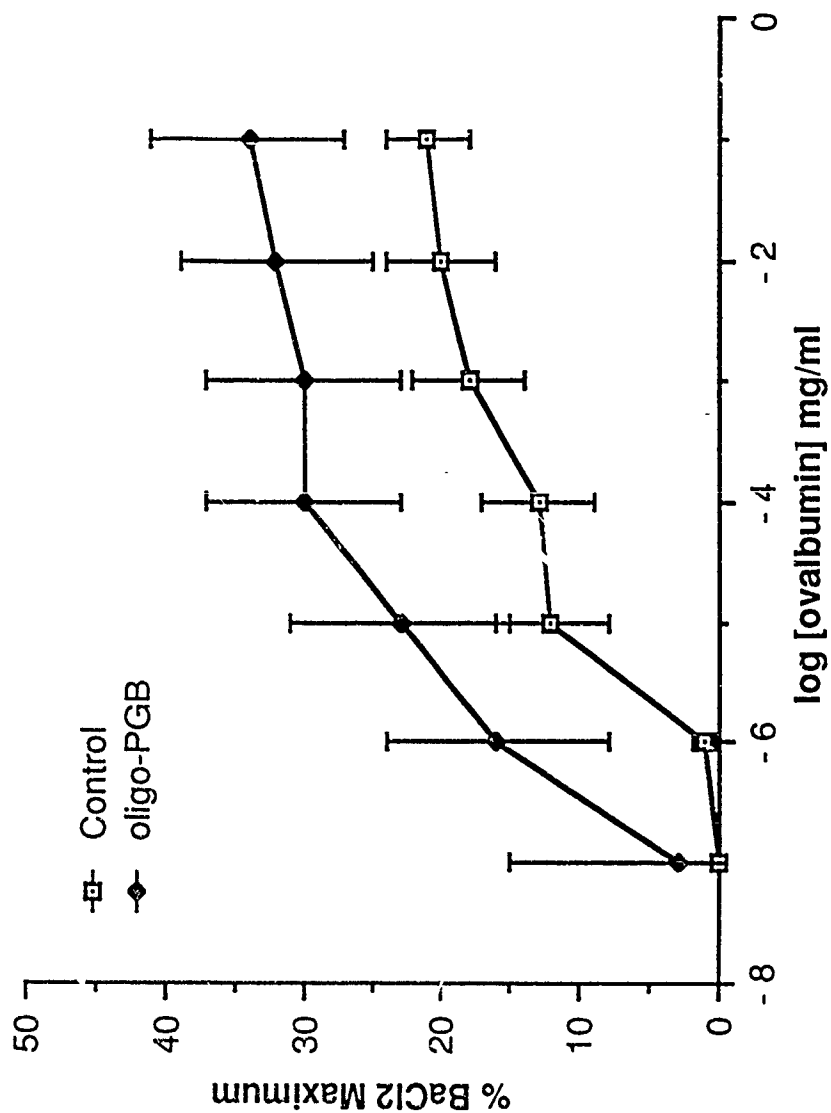


FIG.8 Dose response curve (DRC) to ovalbumin in left bronchi isolated from sensitized guinea pigs and incubated for 24 hours in RPMI 1640 media with or without oligo-PGB (10^{-5} M). Pyrilamine 10^{-6} M were added to the tissue bath 20 minutes before DRC. N=4 in both groups.

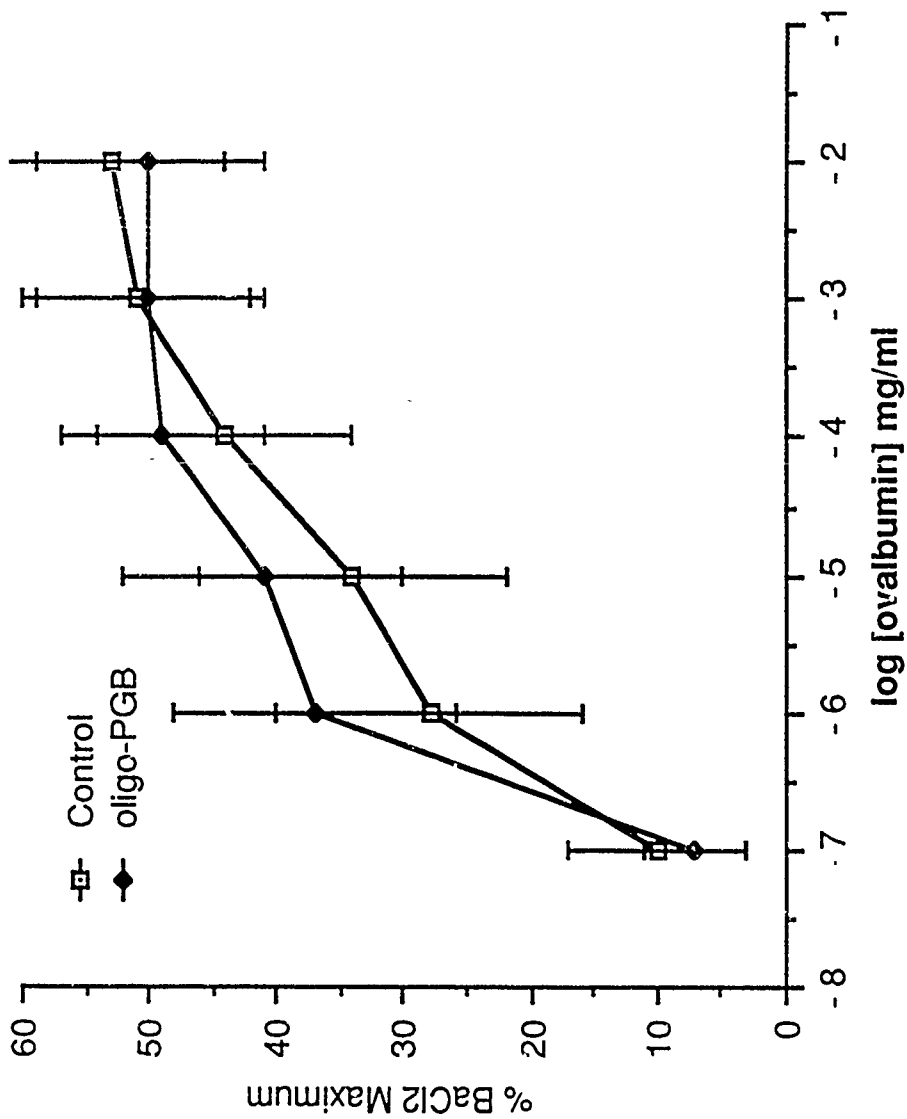


FIG.9 Dose response curve (DRC) to ovalbumin in left bronchi isolated from sensitized guinea pigs and incubated for 24 hours in RPMI 1640 media with or without oligo-PGB (10^{-5} M). Indomethacin 5×10^{-6} M and pyrilamine 10^{-6} M were added to the tissue bath. $N=8$ in both groups.