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Contraction of the Guinea Pig Isolated, Perfused Trachea to Purine and Pyrimidine Agonists

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ABSTRACT

Unlike methacholine and histamine, ATP and uridine 5′-triphosphate (UTP) are more potent contractile agonists when they are applied to the mucosal (intraluminal, IL) surface of the guinea pig perfused trachea than when they are applied to the serosal (extraluminal, EL) surface. The relative contractile activities of a series of purine and pyrimidine compounds were assessed. The order of EL activity was: [2-methythio ATP (2 MeSATP) = adenosine 5′-diphosphate (ADP)] > [adenosine 5′-O-(2-thiodiphosphate) (ADPβS) = ATP = adenosine 5′-O-(3-thiotriphosphate) (ATPγS)] > [(β,γ-imido ATP) (APPNP) = α, β-methylene ATP (APCPP)] > [UTP = uridine 5′-diphosphate (UDP) = inosine 5′-triphosphate (ITP)] > [xanthosine 5′-triphosphate (XTP) β,γ-methylene ATP (APPCP)]. EL adenosine, adenosine 5′-monophosphate, uridine 5′-monophosphate and uridine were weak or inactive. The EL order of activity, therefore, shares some characteristics of P_{2Y} receptors. The order of IL activity was: (ATP = UTP = ITP) > (ATPγS = ADP = APPNP = 2 MeSATP) > (UDP = ADPβS = XTP) > APCPP; the other compounds were weak or inactive. The IL order of activity, therefore, resembled that for P_{2U} or "nucleotide receptors." ATP, APPNP, UTP, UDP, ITP and XTP were more active when added to the IL than after administration to the EL bath; the remaining compounds were similarly active EL and IL, or were more active EL than IL. Greater IL than EL activity of agonists was a property associated with preference for P_{2U}-receptors. The EL and IL activities of the agonists compare favorably with those reported for basolateral and apical stimulation of short circuit current and $[Ca^{++}]_i$ of human respiratory epithelium, and phospholipase C activation of cultured human airway epithelium.

The airway epithelium inhibits the reactivity of airway smooth muscle to most contractile agonists (Fedan *et al.*, 1988). In the guinea pig isolated, perfused trachea preparation, contractions in response to mucosally applied agonists such as methacholine and histamine are smaller than those elicited after the drugs are applied to the serosal surface (Munakata *et al.*, 1989; Fedan and Frazer, 1992). The epithelial diffusion barrier, the production of inhibitory prostanoids and epithelium-derived relaxing factor (EpDRF), and other mechanisms are responsible for reducing reactivity to these agonists after they are applied *via* the mucosal surface. In contrast, we observed that ATP and uridine 5′-triphosphate (UTP) are more potent when applied intraluminally than extraluminally. At least in the case of ATP, greater intraluminal potency was due to a potentiating action of the epithelium (Fedan *et al.*, 1993b).

Responses to extracellular ATP and other adenine nucleotides result from interactions with cell-surface receptors (Head *et al.*, 1983), or P_2-purinoceptors. In the classical view, four P_2-receptors are thought to exist, namely P_{2X}, P_{2Y}, P_{2T} and P_{2Z} (Burnstock, 1990); the latter two are not relevant to the present study and will not be mentioned further. P_{2X}-receptors are characterized by the following order of nucleotide activity: 5′-anhydride oxygen-substituted, nonhydrolyzable analogs ("NHA") > ATP ≥ 2-methylthio ATP (2 MeSATP). P_{2X}-receptors are blocked by $ANAPP_3$ (Hogaboom *et al.*, 1980; Fedan *et al.*, 1985; Fedan and Lamport, 1990). The order of activity at P_{2Y}-receptors is: 2 MeSATP > ATP > NHAs. Evidence has been accumulating that a receptor that interacts with UTP (Urquilla *et al.*, 1978), the P_{2U}-receptor (Seifert and Schultz, 1989; von Kügelgen and Starke, 1990), and a receptor that interacts with both ATP and UTP, the putative "nucleotide receptor" (Brown *et al.*, 1991), may be found in some cells.

Evidence was obtained (Fedan *et al.*, 1993b) that ATP and UTP interact with separate receptors to cause contraction of the perfused trachea. Responses to ATP were abolished by cyclo-oxygenase inhibition with indomethacin, whereas those to UTP were resistant. In the presence of the nonhydrolyzable ATP analog, APPCP, responses to ATP were inhibited,

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ABBREVIATIONS: APPNP, β,γ-imido ATP; APPCP, β,γ-methylene ATP; APCPP, α, β-methylene ATP; ATPγS, adenosine 5′-*O*-(3-thiotriphosphate); ADPβS, adenosine 5′-*O*-(2-thiodiphosphate); 2 MeSATP, 2-methythio ATP; UTP, uridine 5′-triphosphate; UDP, uridine 5′-diphosphate; UMP, uridine 5′-monophosphate; UD, uridine; ITP, inosine 5′-triphosphate; XTP, xanthosine 5′-triphosphate; EL, extraluminal or serosal; IL, intraluminal or mucosal; EpDRF, epithelium-derived relaxing factor

whereas those to UTP were not. Moreover, the adenine nucleotide receptors of the trachea were concluded to have atypical characteristics because APPCP itself did not cause contraction but was a more potent relaxant than ATP (Fedan *et al.*, 1993c). Typically, APPCP is a more potent P_{2X}-receptor contractile agonist, and a less active P_{2Y}-receptor relaxant agonist, than ATP in smooth muscle (Burnstock, 1990).

ATP and UTP stimulate amiloride-sensitive Na^+ absorption and activate Cl^- channels in respiratory epithelium; based on these effects it has been suggested that inhaled ATP or UTP could be useful in cystic fibrosis patients to hydrate the airways (Mason *et al.*, 1991; Knowles *et al.*, 1991). Earlier investigations with adenine and uracil nucleotide analogs have evaluated the P_2-receptors associated with activation of short circuit current, elevation of $[Ca^{++}]_i$, and activation of phospholipase C responses of human respiratory epithelium (Mason *et al.*, 1991; Brown *et al.*, 1991). In Ussing chamber experiments on fresh human nasal epithelium the Cl^- secretory response in response to apical or basolateral application of nucleotides was mediated by receptors whose characteristics resembled neither P_{2X}- nor P_{2Y}-receptors. Moreover, the orders of nucleotide agonist activity after apical or basolateral application were different, *i.e.*, the epithelium was polarized with respect to agonist activity ranking. In cultured CF/T43 cells, stimulation of phospholipase C was concluded to be associated with "nucleotide receptors" (Brown *et al.*, 1991).

In view of the potential utility of inhaled nucleotides as therapy for cystic fibrosis, the present study was conducted to compare the contractile activities of a series of purine and pyrimidine compounds in the isolated, perfused guinea pig trachea preparation. This preparation allows for the separate addition of drugs to the serosal or mucosal surfaces of the trachea. We sought information on the orders of activity of the compounds after extraluminal or intraluminal application of the agonists. Also of interest were the relative activities of the compounds after application *via* the mucosal surface *vis à vis* the serosal surface. The orders of activity for mechanical responses were then compared to the ones obtained for ion transport activation, $[Ca^{++}]_i$ elevation, and phospholipase C activation of human respiratory epithelium (Mason *et al.*, 1991; Brown *et al.*, 1991). Despite some uncertainty in the receptors involved in the human and guinea pig systems, a remarkable similarity in the characteristics of the potency series was seen.

A preliminary account of some of the findings has been given (Fedan *et al.*, 1993a).

Materials and Methods

Perfused trachea preparation. Specific pathogen-free male guinea pigs (English short-hair; 417–685 g; Camm Research Institute, Wayne, NJ) were sacrificed by stunning and were bled. As described in detail (Fedan and Frazer, 1992), a 4-cm-long segment of trachea was removed, cleaned in modified Krebs-Henseleit solution (MKH; bubbled with 95% O_2:5% CO_2) and mounted to a perfusion holder. The holder contained side-hole catheters that became inserted into the lumen from each end after the trachea was mounted. The trachea was restored to its 4-cm length, and the holder was placed into a 9-ml bath of gassed MKH solution (37°C) (the serosal or extraluminal ("EL") bath). The catheters at the inlet and outlet ends of the trachea were connected to the positive and negative sides, respectively, of a differential pressure transducer. The trachea was perfused (34 ml/min) with gassed, recirculating MKH solution (37°C) from a separate reservoir, which with connecting tubing had a volume of 17 ml; the perfusing MKH is referred to as the mucosal or intraluminal ("IL") compartment or bath. The transmural pressure difference was adjusted to zero. Contraction of the trachealis smooth muscle in response to agonists, *i.e.*, decrease in the internal diameter, was given by increases in inlet minus outlet pressure difference (ΔP, in cm H_2O). Each preparation was washed at 15-min intervals by changing the EL and IL baths with fresh MKH solution during a 1-hr equilibration period. The trachea was then challenged with agonists to generate concentration-response curves.

Concentration-response curves. The agonists were added to the EL bath or to the IL perfusing solution in stepwise-increasing, cumulative concentrations. An EL concentration-response curve was obtained first, after which the preparation was washed with fresh MKH for 1.5 hr. An IL curve was then generated. Only one agonist was examined in each trachea.

Methacholine reference contraction. At the conclusion of the IL concentration-response determination the trachea was washed at 15-min intervals for 1 hr. The trachea was then challenged with a maximal concentration of methacholine (10^{-4} M) added to the EL bath to obtain a reference contraction.

IL KCl test. At the plateau of the methacholine reference contraction, 120 mM KCl was administered intraluminally as a functional test of epithelial integrity. IL KCl relaxes intact, contracted trachea by causing EpDRF release, but it contracts trachea from which the epithelium has been damaged or removed (Munakata *et al.*, 1988; Fedan *et al.*, 1990; Fedan and Frazer, 1992). In the present experiments, all of the tracheas relaxed in response to IL KCl.

Analysis of results. Responses were quantified as ΔP in cm H_2O. They were then normalized as a percentage of the methacholine reference contraction (% MCh Contraction). Geometric mean EC_{50} values were derived from least squares analysis of a logit curve fit (SigmaPlot) and are given along with 95% confidence interval (95% C.I.) in parentheses. Other results are expressed as mean ± S.E.; n is the number of separate experiments. Two types of statistical analyses using SAS (Cary, NC) were performed. The first involved comparing the relative potencies of the agonists after they were added to the EL bath or to the IL bath ("within bath" comparison). To test for differences in $-\log[EC_{50}]$ values among the agonists, a modified Fisher's least significant difference procedure (Welch's F-test), followed by Student's t test with the Cochran and Satterthwaite methods for unequal variances, was used. The second type of analysis involved comparisons of the $-\log EC_{50}$ values of the compounds between the EL and IL bath (*i.e.*, "between bath"). The t test with the Cochran and Satterthwaite methods were used for both the overall "between bath" comparison and the "between bath" comparison for each compound. Student's t test for paired samples was used to compare EL and IL maximum responses.

Solutions and reagents. MKH solution contained (mM): NaCl, 113.0; KCl, 4.8; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; and glucose 5.7 (pH 7.4, 37°C).

The following agents were purchased from Sigma Chemical Co. (St. Louis, MO) and dissolved in saline for use: ATP (Na^+ salt, vanadate-free), ADP (Na^+ salt), AMP (Na^+ salt), APPNP (Li^+ salt), APPCP (Na^+ salt), APCPP (Li^+ salt), ATPγS (Li^+salt), ADPβS (Li^+ salt), adenosine hemisulfate, UTP (Na^+ salt), UDP (Na^+ salt), UMP (Na^+ salt), uridine, ITP (Na^+ salt), XTP (Na^+ salt), and methacholine (acetyl-β-methylcholine chloride). 2 MeSATP (Li^+ salt) was purchased from Research Biochemicals Inc. (Natick, MA) and dissolved in saline.

Results

ATP was more potent intraluminally than extraluminally (fig. 1; table 1), confirming our earlier findings (Fedan *et al.*, 1993b). Unlike ATP, IL ADP was equipotent, not more potent, with EL ADP. For ATP and ADP the EL and IL maximum responses were not different. Compared to ATP and ADP, AMP and ADO had little or no activity. Although not efficacious, IL AMP and EL ADO possessed moderate potency.

The results obtained with the P_{2X}-purinoceptor selective adenine nucleotides, APPNP, APPCP and APCPP, are shown

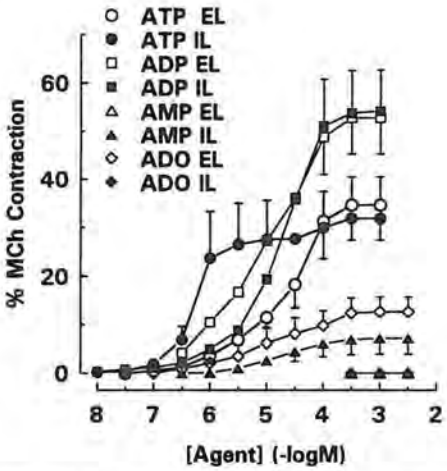

Fig. 1. Comparison of EL (open symbols) and IL (filled symbols) concentration-response curves for adenine nucleotides and adenosine in isolated, perfused guinea pig trachea. The contractile responses were normalized as a percentage of the reference contraction to 10^{-4} M methacholine (MCh) obtained at the end of the experiment.

in figure 2 and table 1. Also shown is the activity of ATPγS, an agonist that in smooth muscle induces contractions that appear to involve transduction by an ectokinase (Lamport-Vrana *et al.*, 1991). Like ATP, APPNP was more active intraluminally than extraluminally. In confirmation of our earlier findings (Fedan *et al.*, 1993b), EL and IL APPCP had virtually no activity. APCPP was not particularly potent in either bath. ATPγS was equiactive in both compartments, with a potency comparable to EL ATP.

Concentration-response curves for the P_{2Y}-purinoceptor-selective agonists are shown in figure 3. Both ADPβS and 2 MeSATP were clearly less active in the IL bath than in the EL bath; this appeared as a lesser potency (*e.g.*, ADPβS) or as a smaller IL maximum response (*e.g.*, 2 MeSATP) (table 1).

Except for EL XTP, purine nucleotide agonists were quite efficacious after both extraluminal and intraluminal application (fig. 4, table 1). ITP and XTP were more active intraluminally compared to extraluminally, both in terms of EC_{50} values and in terms of maximum contractile responses.

The activities of uracil nucleotides and uridine are summarized in figure 5 and table 1. UDP had less activity than UTP after EL and IL application. However, both UTP and UDP were more active in the IL bath than in the EL bath. The results obtained with UTP confirm our earlier findings (Fedan *et al.*, 1993b). UMP was devoid of activity. IL uridine induced weak responses in one trachea.

The results of statistical analysis of differences in the potencies of the compounds in the EL and IL compartments are shown in table 2. Compounds with weak activity were not included in this analysis. When ranked from high to low potency, using the EC_{50} values in table 1, there were no differences in the values of adjacent compounds in either the EL or the IL bath. This was because the decrements in potency between neighboring compounds was usually small. The bottom of table 2 shows a diagram of clusters of compounds, within which there were no statistical differences in EC_{50} values (only those agents for which the EC_{50} could be calculated are shown). The horizontal bars indicate the groupings in which there were no differences in the EC_{50} values. That is, compounds that do not lie above the same continuous line are different statistically. Several clusters of statistical identity could be described (bottom of table 2), some of which were in continuity with adjacent clusters (see EL bath). From a conservative statistical standpoint, the following rankings emerged: for the EL bath, (2 MeSATP = ADP) > (UTP = UDP = ITP = XTP); for the IL bath, (ATP = UTP = ITP) > ATPγS > (ADPβS = XTP = APCPP). Using the statistical analysis as a guide for interpretation, and assuming that a difference in EC_{50} values of ~2-fold or greater signifies a biologically meaningful difference, we interpret the orders of activity to be those shown in figure 6.

TABLE 1

EL and IL potencies and maximum contractile responses in isolated, perfused guinea pig trachea

95% confidence interval in parentheses.

Agonist (*n*)[b]	EC_{50}		EC_{50} Ratio (IL/EL)	Maximum Response[a]	
	EL	IL		EL	IL
	μM				
ATP (10)	20.0 (1.1–3.6)	1.6 (0.8–3.3)*	0.1106 ± 0.0250	34.9 ± 5.9	31.9 ± 4.5
ADP (4)	9.8 (3.7–25.5)	18.1 (6.4–51.0)	2.1106 ± 0.5386	52.7 ± 7.5	54.1 ± 8.5
AMP (4)	—	19.8 (14.9–26.2)	—	0.0	7.2 ± 3.4
ADO (4)	19.2 (3.7–99.6)	—	—	12.7 ± 3.1	0.0
APPNP (4)	84.6 (30.1–237.5)	19.0 (9.3–38.6)*	0.3504 ± 0.1156	15.9 ± 2.4	28.0 ± 4.3
APPCP (4)	104.6 (3.7–2951.9)	—	—	3.8 ± 3.1	1.6 ± 1.6
APCPP (5)	123.2 (43.9–345.7)	302.3 (130.8–698.7)	4.8652 ± 2.9660	24.6 ± 12.6	11.1 ± 4.7
ATPγS (5)	25.1 (13.7–45.8)	12.9 (4.7–35.8)	0.6252 ± 0.1654	33.8 ± 7.8	24.6 ± 6.2
2 MeSATP (4)	6.3 (1.6–24.8)	20.1 (4.5–90.3)	9.4802 ± 7.5625	47.1 ± 12.0	18.9 ± 2.3*
ADPβS (5)	18.5 (10.6–32.2)	95.7 (32.8–278.9)**	6.8314 ± 2.1052	40.7 ± 5.5	24.9 ± 7.5
UTP (9)	202.2 (95.3–429.0)	1.8 (0.6–5.6)*	0.0472 ± 0.0302	12.9 ± 2.4	25.8 ± 9.3
UDP (8)	366.0 (311.4–430.2)	87.2 (63.6–119.4)*	0.2605 ± 0.0439	9.8 ± 3.1	20.5 ± 5.1*
UMP (4)	—	—	—	0.0	0.0
UD (4)	—	152.3	—	0.0	0.6
ITP (4)	345.0 (300.0–397.3)	2.7 (1.4–4.9)*	0.0089 ± 0.0028	29.6 ± 12.7	54.3 ± 9.0**
XTP (4)	502.5 (385.7–654.6)	98.7 (43.0–226.5)*	0.2556 ± 0.1182	2.7 ± 0.7	34.8 ± 3.9**

[a] % of EL MCh(10^{-4} M)-induced contraction.

[b] The *n* values shown in this column are the number of attempted experiments. For some agonists the number of preparations that gave adequate responses to permit EC_{50} and IL/EL EC_{50} ratio calculations was less than this number, as follows: AMP, 0/4 for EL, 3/4 for IL; ADO, 4/4 for EL, 0/4 for IL; APPCP, 2/4 for EL, 1/4 for IL; UMP, 0/4 for EL, 0/4 for IL, UD, 0/4 for EL, 1/4 or IL. Dashed lines indicate that EC_{50} values or ratios could not be calculated due to insufficient data.

* IL < EL, $P < .05$.

** IL > EL, $P < .05$.

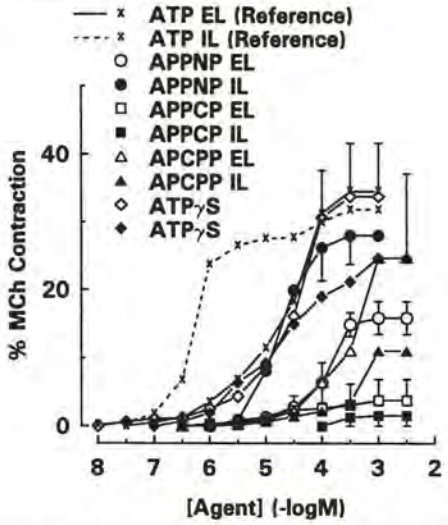

Fig. 2. Comparison of EL (open symbols) and IL (filled symbols) concentration-response curves for phosphate chain-substituted adenine nucleotides in isolated, perfused guinea pig trachea. Curves for the P_{2X}-purinoceptor-selective, nonhydrolyzable ATP analogs (APPNP, APCPP, APPCP) and ATPγS are plotted in comparison with the EL and IL ATP reference curves (mean values only).

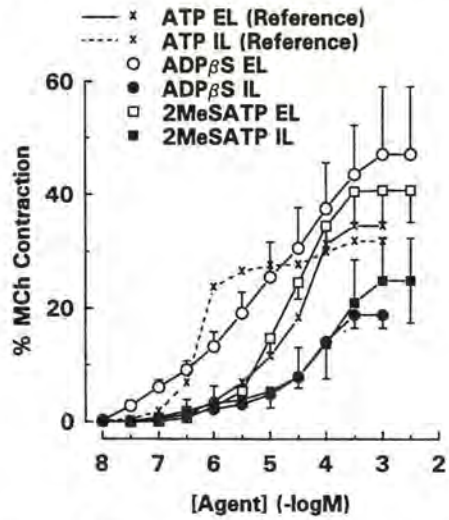

Fig. 3. Comparison of EL (open symbols) and IL (filled symbols) concentration-response curves for P_{2Y}-purinoceptor-selective adenine nucleotides in isolated, perfused guinea-pig trachea. The EL and IL ATP reference curves (mean values only) are shown for comparison.

Discussion

The nucleotides and nucleosides examined in this study differed with respect to 1) their order of potency in the EL bath, 2) their order of potency in the IL bath, and 3) their relative activities in the EL bath compared to the IL bath. It is clear that the orders of EL and IL activity (fig. 6) for the sixteen compounds studied were different. Inasmuch as APPNP, APPCP and APCPP were less active than ATP in both the EL and the IL baths, these NHAs probably are not stimulating classical P_{2X}-purinoceptors to cause contraction.

The order of activity in the EL bath (fig. 6) could be viewed as resembling most closely, but not exactly, that for the P_{2Y}-purinoceptor, *i.e.*, 2 MeSATP > ATP > NHAs. The difference in activity between 2 MeSATP and ATP (and agonists with similar activity in these clusters) was not large, which is atypical for P_{2Y}-purinoceptors. For example, in lung parenchymal strips, Satchell (1990) reported that 2 MeSATP was ~1000-fold more potent than ATP. It could be argued that the differences within the five most active agonists are not biologically important, and that the first relevant difference in EL bath activity occurred between the first five analogs and APPNP along with APCPP (table 1, fig. 6). The results would then suggest that the EL receptor shows no preference for the P_{2Y}-selective agonists ADPβS and 2 MeSATP, or for ATP, ADP or ATPγS.

The order of activity in the IL bath (fig. 6) was not in agreement with that for classical P_{2Y}-purinoceptors. However, UTP was very active in the IL bath. Therefore, the responses could have involved a P_{2U}-receptor or putative "nucleotide receptor" (Brown *et al.*, 1991). The activity ranking profile for the putative nucleotide receptor is thought to be: UTP = ATP > ADP > 2 MeSATP. In the IL bath, while ATP and UTP

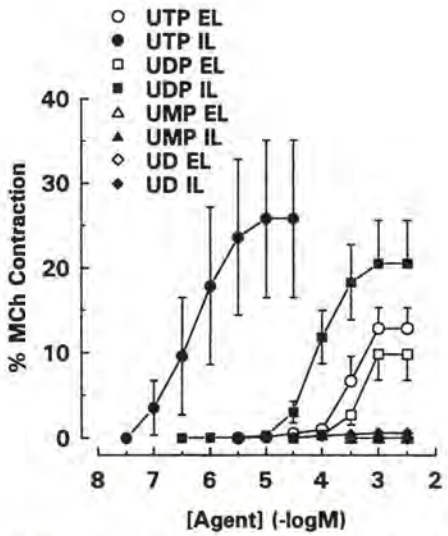

Fig. 4. Comparison of EL (open symbols) and IL (filled symbols) concentration-response curves for uracil nucleotides and uridine in isolated, perfused guinea pig trachea.

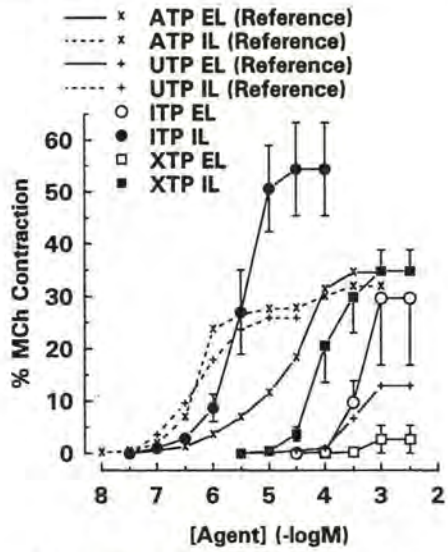

Fig. 5. Comparison of EL (open symbols) and IL (filled symbols) concentration-response curves for ITP and XTP in isolated, perfused guinea pig trachea. Reference curves for EL and IL ATP and UTP (mean values) are indicated for comparison.

TABLE 2

Comparison of EC_{50} values in the EL and IL baths: probabilities from paired EC_{50} testing

Extraluminal Bath

	ATP	ADP	APPNP	APPCP	ATPγS	2MeSATP	ADPβS	UTP	UDP	ITP	XTP
ATP	—	0.261	0.078	0.099	0.609	0.200	0.850	0.000	0.000	0.000	0.001
ADP		—	0.029	0.037	0.161	0.629	0.310	0.002	0.004	0.005	0.002
APPNP			—	0.974	0.118	0.028	0.070	0.333	0.112	0.123	0.085
APCPP				—	0.145	0.032	0.088	0.341	0.123	0.135	0.095
ATPγS					—	0.142	0.488	0.001	0.001	0.001	0.003
2MeSATP						—	0.226	0.008	0.010	0.010	0.006
ADPβS							—	0.000	0.000	0.000	0.003
UTP								—	0.165	0.206	0.149
UDP									—	0.600	0.621
ITP										—	0.532
XTP											—

Intraluminal Bath

	ATP	ADP	APPNP	APPCP	ATPγS	2MeSATP	ADPβS	UTP	UDP	ITP	XTP
ATP	—	0.008	0.001	0.000	0.011	0.036	0.000	0.864	0.000	0.314	0.001
ADP		—	0.945	0.011	0.657	0.916	0.062	0.014	0.049	0.025	0.066
APPNP			—	0.007	0.564	0.949	0.045	0.005	0.017	0.007	0.049
APCPP				—	0.005	0.038	0.227	0.000	0.121	0.001	0.139
ATPγS					—	0.652	0.029	0.027	0.019	0.039	0.030
2MeSATP						—	0.151	0.042	0.150	0.072	0.165
ADPβS							—	0.000	0.876	0.001	0.844
UTP								—	0.000	0.564	0.000
UDP									—	0.000	0.929
ITP										—	0.005
XTP											—

EL: 2MeSATP ADP ADPβS ATP ATPγS APPNP APCPP UTP UDP ITP XTP

IL: ATP UTP ITP ATPγS ADP APPNP 2MeSATP UDP ADPβS XTP APCPP

were equipotent, ADP and 2 MeSATP were equiactive, and ADPβS was weaker than the latter two analogs. Therefore, the agreement with the classification for nucleotide receptors was not exact. If uracil nucleotides and nucleotide receptor agonists are omitted from the ranking, on the assumption that ATP and UTP act on different receptors (Fedan *et al.*, 1993b), then the following order of activity results: ATP > (ATPγS = ADP = APPNP = 2 MeSATP) > ADPβS > APCPP. This ranking is inconsistent with P_{2Y}-receptor characteristics.

Despite the deviations of activity rankings from the classical patterns, the EL and IL orders of contractile activity in the perfused trachea compare well with the rankings obtained for the stimulatory effects of the compounds on short circuit current (I_{sc}) and $[Ca^{++}]_i$ in human nasal epithelium, after their apical (*i.e.*, IL) and basolateral (*i.e.*, EL) application (Mason *et al.*, 1991) (fig. 7). One discrepancy in the EL (basolateral) compartment was UTP, which was among the most potent compounds in human epithelium, but which was weakly active in the guinea pig trachea. In the IL (apical) bath the agreement between the findings of Mason *et al.* (1991) and the present study was very close; the results differed in that we observed ATPγS to be equiactive with 2 MeSATP, whereas ATPγS was more potent than 2 MeSATP in nasal epithelium. We also observed IL ITP to be very potent, but Mason *et al.* (1991) found ITP to weakly elevate $[Ca^{+}]_i$. The observation made by Mason *et al.* (1991) that APCPP was more potent than APPCP was also seen in our experiments.

The IL order of nucleotide activity in the trachea was in reasonable agreement with that for activation of phospholipase C in human cultured airway epithelial (CF/T43) cells (Brown *et al.*, 1991) (fig. 7), but there was no similarity with the EL order of activity. Some other differences in the perfused trachea (IL bath) and CF/T43 cell results were as follows: 1) ATP and UTP were equipotent in trachea, whereas UTP was more potent than ATP in activating phospholipase; 2) ITP was among the most potent contractile agonists, whereas ITP had moderate potency in the cultured cells; and 3) ATPγS and ADP were equiactive and of fairly high potency in trachea, whereas ADP was a weak agonist in cultured cells. On balance, the effects of the nucleotides in the trachea are in much better agreement with the effects of the compounds on I_{sc} (in which it was concluded that the receptors for nucleotides are different on the apical and basolateral surfaces of epithelial cells) than for phospholipase C activation (in which it was proposed that a single receptor, the nucleotide receptor, mediated the response). A loss of apical membrane-basolateral membrane receptor polarization in cultured cells might contribute to these differences.

Other considerations are pertinent to the interpretation of the present findings. For example, we have already observed that ATP and UTP cause contraction *via* separate receptors (Fedan *et al.*, 1993b). Second, IL-applied agonists undoubtedly affected basolateral ion channels in epithelial cells as they diffused through the mucosa to the smooth muscle. The recep-

EL:

(2MeSATP = ADP) > (ADPβS = ATP = ATPγS) > (APPNP = APCPP) > (UTP = UDP = ITP) > XTP ➤ ADO > APPCP > AMP

IL:

(ATP = UTP = ITP) > (ATPγS = ADP = APPNP = 2MeSATP) > (UDP = ADPβS = XTP) > APCPP > AMP > ADO

Fig. 6. Estimation of the EL and IL orders of activity. These rankings take into account the statistical analysis of EC_{50} values (where they could be obtained) and the ability of the compounds to evoke a response.

EL or basolateral:							
Trachea ΔP EL	(2MeSATP = ADP) >	(ADPβS = ATP = ATPγS) >	(APPNP = APCPP) >	(UTP = UDP = ITP) >	XTP ➤ APPCP		
Nasal I_{sc}	2MeSATP >	UTP >	ATP > ATPγS >	APCPP >		APPCP	
IL or apical:							
Trachea IL ΔP	(ATP = UTP = ITP) >	(ATPγS =	ADP =	APPNP = 2MeSATP) >	(UDP = ADPβS = XTP) >	APCPP	
Nasal I_{sc}	UTP ≥ ATP >	ATPγS >		2MeSATP >	ADPβS ➤	APCPP	
Nasal ↑ $[Ca^{2+}]_i$:	UTP ≥ ATP >	ATPγS >		2MeSATP >	ADPβS >	ITP	
Cultured Epi ↑ PLC:	UTP > ATP >	ATPγS > ITP > ADP ➤		2MeSATP =	ADPβS >	APCPP	

Fig. 7. Comparison of the EL and IL orders of contractile activity in perfused trachea with those for short circuit current (I_{sc}), elevation in $[Ca^{++}]_i$, and phospholipase C (PLC) activation in human respiratory epithelium. The orders of activity for I_{sc} were from the study of Mason *et al.* (1991) on nasal epithelium. The results for PLC activation of cultured human epithelium are those of Brown *et al.* (1991). The compounds that are underlined are "out of place" with respect to the rankings for trachea.

tors for nucleotides coupled to ion channels are different on the apical and basolateral surfaces of respiratory epithelial cells (Mason *et al.*, 1991). It is also conceivable that EL-applied compounds stimulated receptors on the apical surface of the epithelial cells. Stimulation of one or more of these epithelial receptors is responsible for the greater IL potency of ATP (Fedan *et al.*, 1993b). Thus, interactions of the nucleotides with multiple receptors on the epithelial apical and basolateral membranes, and receptors on the smooth muscle, may have contributed to the activity rankings observed in this study.

Aside from their location in the EL and IL activity rankings, the compounds examined in our experiments differed with respect to their relative activities in the EL bath *vis á vis* the IL bath. ATP, APPNP, UTP, UDP, ITP and XTP were significantly more active IL than EL; ADP, APCPP and ATPγS were equiactive in the EL and IL baths; and 2 MeSATP and ADPβS were less active in the IL bath compared to the EL bath. When ranked in order of declining IL *vs.* EL selectivity, *i.e.*, increase in the IL/EL EC_{50} ratio [ITP (0.01) > UTP (0.05) > ATP (0.11) > XTP (0.26) = UDP (0.26) > APPNP (0.35)], the sequence suggests that P_{2U} purinoceptor- or nucleotide receptor-selective agonists as a class are more potent intraluminally than extraluminally, even in the face of the epithelial diffusion barrier.

There are reasons why the assignment of P_2-receptor subtypes in the perfused trachea should be considered provisional and in need of future investigation. First, the IL order of activity would be influenced by the relative permeabilities of the nucleotides across the mucosa. Second, the nucleotides used in this study that did not contain 5′-phosphate anhydride substitutions are potentially hydrolyzable and were probably degraded to varying degrees by ecto-phosphohydrolases present on one or more of the numerous cell types present in the airway wall. The degree to which this metabolism influenced the orders of potency in the EL and IL baths is not known at present. The likelihood of metabolism would be seemingly greater after the agonists were added to the IL bath, inasmuch as the epithelium is known to metabolize other agonists (see Fedan and Frazer, 1992). The fact that ATP was more active than APPCP, APCPP and APPNP in both compartments would suggest that nucleotide breakdown has little effect on nucleotide activity. However, 5′-anhydride oxygen substitution may alter receptor selectivity in the perfused trachea. For example, APPCP (but not UTP) relaxed the pre-contracted trachea, as did higher concentrations of ATP; APPCP was more potent than ATP, and it was more active in the EL bath than in the IL bath (Fedan *et al.*, 1993c); in contrast, ATP and APPCP both relax guinea pig aorta, but APPCP is *less* potent than ATP (Hourani *et al.*, 1986.) Thus, if the other NHAs produce a relaxant signal in the perfused trachea, the orders of contractile activity would be affected. Tracheal relaxation responses to ATP and APPCP were not mediated by adenosine receptors, because they were not blocked by 8-phenyltheophylline (Fedan *et al.*, 1993c). The definition of the receptors involved in contractile and relaxation responses to nucleotides administered extraluminally and intraluminally will be aided by successful blockade of the responses with specific antagonists.

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