Prostaglandin involvement in lung C-fiber activation by substance P in guinea pigs

Dale R. Bergren

Department of Biomedical Sciences, School of Medicine, Creighton University, Omaha, Nebraska

Submitted 4 October 2005; accepted in final form 25 January 2006

Bergren, Dale R. Prostaglandin involvement in lung C-fiber activation by substance P in guinea pigs. J Appl Physiol 100: 1918–1927, 2006. First published February 2, 2006; doi:10.1152/japplphysiol.01276.2005.—Airway hyperresponsiveness is a cardinal feature of asthma. Lung C-fiber activation induces central and local defense reflexes that may contribute to airway hyperresponsiveness. Initial studies show that substance P (SP) activates C fibers even though it is produced and released by these same C fibers. SP may induce release of other endogenous mediators. Bradykinin (BK) is an endogenous mediator that activates C fibers. The hypothesis was tested that SP activates C fibers via BK release. Guinea pigs were anesthetized, and C-fiber activity (FA), pulmonary insufflation pressure (PIP), heart rate, and arterial blood pressure were monitored before and after intravenous injection of capsaicin (Cap), SP, and BK. Identical agonist challenges were repeated after infusion of an antagonist cocktail of des-Arg9-[Leu8]-BK (10⁻⁸ M, B₁ antagonist), and HOE-140 (10⁻⁴ M, B₂ antagonist). After antagonist administration, BK increased neither PIP nor FA. Increases in neither PIP nor FA were attenuated after Cap or SP challenge. In a second series of experiments, Cap and SP were injected before and after infusion of indomethacin (1 mg/kg i.v.) to determine whether either agent activates C fibers through release of arachidonic acid metabolites. Indomethacin administration decreased the effect of SP challenge on FA but not PIP. The effect of Cap on FA or PIP was not altered by indomethacin. In subsequent experiments, C fibers were activated by prostaglandin E₂ and F₂α. Therefore, exogenously applied SP stimulates an indomethacin-sensitive pathway leading to C-fiber activation.

C-fiber activity

MECHANISMS OF ASThma remain elusive. However, both clinical and animal studies suggest that activation of certain pulmonary sensory receptors contribute to airway hyperresponsiveness through both central and local reflexes (2, 29, 32). Of the three categories of pulmonary sensory receptors, C-fiber endings are thought to contribute to symptoms of asthma by inducing defense reflexes (2, 29). C-fiber activation induces inflammatory responses that include bronchoconstriction, increased mucous production, and vasodilation and edema formation.

C fibers are known to produce and release substance P (SP) on activation (28). Preliminary studies showed that SP activates lung C fibers (5). C-fiber activation by SP could be direct, indirect, or varying degrees of both. If activation were direct, then this would represent an autocrine feedforward mechanism. If activation were indirect, then this would involve release of one or more endogenous mediator(s).

Few endogenous agents are known to directly activate C fibers. Bradykinin (BK) is perhaps the most potent and dominant endogenous agent that activates C fibers (3, 4, 15, 22). BK is believed to activate sensory nerves by binding to its B₂ receptor in the cell membrane (23). This may activate the cyclic GMP system to initiate action potentials. Therefore, the initial series of experiments tested the hypothesis that SP causes generation of BK, which then directly activates airway C fibers.

METHODS

Animals. The Creighton University Animal Use Committee approved the protocol used in the present study. Twenty Hartley guinea pigs (Harlan, Minneapolis, MN) were housed in the Creighton University School of Medicine Animal Resource Facility with food and water ad libitum.

Surgical preparations. Guinea pigs were deeply anesthetized with pentobarbital sodium (50–75 mg/kg ip, Astra, Arcadia, CA). Surgical anesthesia was maintained throughout the experiment with supplemental injections of one-quarter of the original dose at ~2-h intervals.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Testing spinal and corneal reflexes assessed the level of anesthesia. Additional pentobarbital sodium was administered intraperitoneally if either reflex was present. Skeletal muscle blockade was not utilized at any time during the study.

Guinea pigs were placed in the supine position on an operating table. A heated water bath with a circulation pad (Gaymar, Orchard Park, NY) maintained normal body temperature. An anterior, midline incision was made in the neck. The right carotid artery was cannulated. The cannula was connected to a pressure transducer (model P23XL, Spectromed, Oxnard, CA) attached to a polygraph (Grass 7D, Quincy, MA) to monitor arterial blood pressure and heart rate. The right jugular vein was cannulated for the purpose of drug injection. The trachea was cannulated. The cannula was connected to a ventilator (rodent ventilator model 683, Harvard Apparatus). A pressure transducer (model P23XL, Spectromed) connected by a T tube in the expiratory line from the tracheal cannula to the ventilator monitored pulmonary insufflation pressure (PIP). The ventilator was set at 70 cycles/min with a stroke volume of 0.75 ml/100 mg body wt. The chest was opened by a midsternum incision to provide access to the lung parenchyma. This opening was used to establish that the receptive field of the sensory receptor of the nerve fiber being studied was within the lungs by gentle probing with a cotton pledget. The expiratory line of the ventilator was placed under 3-5 cm of water to maintain normal functional residual capacity. The lungs were frequently inflated to three times tidal volume to maintain a consistent pulmonary compliance and volume history.

Nerve recording. The left vagus nerve was exposed in the neck and then cut near the nodose ganglion. The skin surrounding the incision was elevated to form a trough that was filled with mineral oil to prevent drying of the nerve. The distal end of the nerve was freed from surrounding tissue and placed on a dissection platform. Using a dissecting microscope, nerve fibers were dissected from the nerve bundles and placed on a recording electrode held by a micromanipulator. The signal from the electrode was relayed to a preamplifier (Tektronix AM502, Beaverton, OR) connected to an oscilloscope (Tektronix 5228). From the oscilloscope, the signal was relayed to an audiomonitor (AM8, Grass) and to a computerized data-acquisition system (Power Lab, Castle Hill, Australia) that also recorded PIP, arterial blood pressure, and heart rate.

![Fig. 2](http://jap.physiology.org/) Capsaicin challenges (1 µg iv) before (A) and after (B) administration of BK 1 and 2 receptor antagonists (see METHODS for details). The arrow is time at which loading and flushing of agonist are initiated in this and subsequent figures. A larger C fiber becomes more active in B. Note that neither fiber is activated by stepwise lung inflation at the end of each record. The same preparation is shown in Fig. 3 for BK challenge and Fig. 4 for SP challenge, as in this series of experiments 1 C fiber was challenged with all 3 agonists before and after BK antagonist administration. HR, heart rate; ABP, arterial blood pressure; B/M, beats/min.
Lung C fibers were initially identified by activation by capsaicin challenge. The exogenous agent capsaicin has long been used as a tool to activate and identify lung C fibers (13, 14, 19). C-fiber activation by capsaicin is direct via the membrane-bound vanilloid or VR1 receptor (10). Other variables used to verify that the fiber being studied was a lung C fiber were a conduction velocity indicative of nonmyelinated fibers, weak response to lung hyperinflation compared with either slowly or rapidly adapting pulmonary stretch receptors, and a receptive field located within the lungs (3, 4). To determine the fiber’s conduction velocity, stimulating electrodes were placed on the vagus nerve near its emergence from the thorax. The distance between the stimulating and recording electrodes was measured (~20 mm). A signal generated by a stimulator (model S88, Grass) triggered a sweep of the oscilloscope. The conduction velocity was calculated by dividing the distance between the two electrodes by the time between the stimulus artifact and the onset of the action potential of the fiber. All C fibers of this study had conduction velocities of ~2.3 m/s. Fiber activity was monitored as the lungs were stepwise inflated to four times tidal volume and to a constant pressure inflation of 30 cmH2O for 5 s to verify that the fiber was neither a slowly or rapidly adapting stretch receptor.

Agents. All agents were purchased from Sigma Chemical (St. Louis, MO) with the exception of pentobarbital sodium, which was purchased from Anpro Pharmaceuticals (Arcadia, CA). The experiments were terminated with an intravenous overdose of the anesthetic agent.

Protocol. Agonist challenges (1 μg of capsaicin, BK, or SP in 0.1 ml) were loaded into the venous catheter (0.15-ml dead space). These doses were determined from results of preliminary experiments and previous studies (3, 4). The catheter was then flushed with 0.2 ml of 0.9% saline solution. Ten minutes separated agonist challenges. BK receptor antagonism consisted of a 0.4-ml cocktail infusion of HOE-140 (10^-4 M) and des-Arg^9-[Leu^8]-BK (10^-3 M). Ten minutes after BK receptor antagonist administration, identical challenges of the agonists were repeated, although the order of agonist injection was reversed. The rationale was that BK was injected first to ensure effective blockade and capsaicin last to ensure receptor viability. The

Fig. 3. BK challenges (1 μg iv) before (A) and after (B) administration of BK B1 and B2 receptor antagonists (see METHODS for details). Stepwise lung inflation at the end of each record rules out the presence of slowly or rapidly adapting pulmonary stretch receptors.
full protocol was completed in eight guinea pigs while studying one C fiber per animal.

In a separate series of experiments, capsaicin and SP challenges were performed in eight guinea pigs (1 C fiber/animal) before and 10 min after indomethacin administration (1 mg/kg iv). In a final series of experiments, in four guinea pigs, prostaglandin E2 and then F2α (10 and 20 μg in 0.1 ml, respectively) were administered intravenously. Because no antagonists were used in this series of experiments, two to three C fibers per guinea pig were challenged with both prostaglandins.

Statistical analysis. PIP was determined from the pressure swing of a single ventilation cycle. Base PIP was the peak pressure within 60 s immediately before mediator challenge. Peak PIP was the peak pressure within 60 s immediately after mediator challenge. Fiber activity was the peak activity averaged during a consecutive 6-s period within 60 s immediately before (baseline fiber activity) and within 60 s immediately after mediator challenge (peak fiber activity). Data are presented as means ± SE.

To test the hypothesis that an agonist works through a pathway leading to another agonist release, we compared a variable’s absolute difference from baseline to its peak without antagonist administration (Δ1) to a variable’s absolute difference from baseline to its peak with prior antagonist administration (Δ2). Because all measurements are on the same animal, this is repeated measures. Therefore, we used a two-sided paired t-test to test the null hypothesis that the mean of Δ1 equals the mean of Δ2. To test the null hypothesis that antagonist administration does not alter baseline, we again employed the paired t-test of the raw variable values before vs. after antagonist administration.

RESULTS

The profile of C-fiber activation and PIP after capsaicin, BK, and SP challenges is presented in Fig. 1. At the dosage of 1 μg of capsaicin, C-fiber activation was rapid in onset and short in duration (Fig. 2). The onset of activation often occurred before detectable increases in PIP. C-fiber activation by both BK and SP was often delayed but longer in duration compared with that of capsaicin (Figs. 3 and 4). These data suggest that C-fiber...
activation by BK and SP may involve secondary mediators. SP is produced within C fibers (28). Therefore, the hypothesis was tested that SP induces generation or release of endogenous mediators that activate C fibers. BK is an endogenous mediator that activates C fibers directly (3, 15). To test the hypothesis that SP activation of C fibers is at least in part mediated by BK, a cocktail of BK B1 and B2 receptor antagonists was administered between identical agonist injections.

Capsaicin challenge decreased arterial blood pressure, increased PIP, and activated C fibers (Fig. 2A and Table 1). BK B1 and B2 receptor blockade failed to attenuate either the increase in C-fiber activity or PIP induced by capsaicin (Fig. 2B). Mean C-fiber activity and PIP from these challenges are presented in Fig. 5, A and B. Capsaicin increased C-fiber activity from baseline before and after BK receptor blockade 5.7 ± 2.0 and 7.3 ± 1.1 impulses/s, respectively. These means were not statistically separable. The change in PIP from baseline before and after BK receptor blockade was 9.8 ± 3.3 to 19.2 ± 3.5 cmH2O, respectively (P = 0.03).

BK challenge decreased arterial blood pressure, increased PIP, and activated C fibers (Fig. 3A and Table 1). BK B1 and B2 receptor blockade effectively attenuated C-fiber activation and the increase PIP induced by BK challenge (Fig. 3B). Mean C-fiber activity and PIP from these challenges are presented in Fig. 5, C and D. The change in C-fiber activity was 4.4 ± 0.6 before and 0.5 ± 0.2 impulses/s after BK receptor blockade (P = 0.0025). The change in PIP was 7.5 ± 2.0 before and 0.6 ± 0.3 cmH2O after BK receptor blockade (P = 0.012).

SP challenge decreased arterial blood pressure, increased PIP, and activated C fibers (Fig. 4A and Table 1). BK B1 and B2 receptor antagonism failed to attenuate either the increase in C-fiber activity or PIP induced by SP challenge (Figs. 4B and 5, E and F). SP increased C-fiber activity from baseline 5.5 ± 1.1 before and 4.7 ± 1.2 impulses/s after BK receptor blockade. The change in PIP from baseline before and after BK receptor blockade was 16.8 ± 2.0 and 22.0 ± 4.8 cmH2O, respectively (P > 0.05).

Because C-fiber activation by SP was not attenuated by BK receptor blockade, other mediators known to activate C fibers were considered as possible agents mediating SP-related C-fiber activation. Prostaglandin E2 and F2α activate canine lung C fibers (12). Therefore, the hypothesis was

---

Table 1. Effect of intravenous capsaicin, bradykinin, and substance P challenge in anesthetized guinea pigs

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time</th>
<th>Capsaicin</th>
<th>Bradykinin</th>
<th>Substance P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>Base</td>
<td>211.0 ± 6.8</td>
<td>207.8 ± 6.9</td>
<td>211.4 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>Peak</td>
<td>186.5 ± 8.6</td>
<td>197.1 ± 9.7</td>
<td>192.0 ± 7.5</td>
</tr>
<tr>
<td>Systolic ABP, mmHg</td>
<td>Base</td>
<td>49.3 ± 2.1</td>
<td>53.1 ± 3.7</td>
<td>52.9 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>Peak</td>
<td>38.0 ± 1.9</td>
<td>36.5 ± 3.9†</td>
<td>34.9 ± 2.2§</td>
</tr>
<tr>
<td>Diastolic ABP, mmHg</td>
<td>Base</td>
<td>29.6 ± 1.8</td>
<td>37.8 ± 1.4</td>
<td>33.2 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>Peak</td>
<td>19.4 ± 1.3§</td>
<td>19.8 ± 2.0‡</td>
<td>17.9 ± 1.3§</td>
</tr>
<tr>
<td>PIP, cmH2O</td>
<td>Base</td>
<td>6.2 ± 0.3</td>
<td>4.6 ± 0.6</td>
<td>5.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Peak</td>
<td>17.6 ± 2.1§</td>
<td>11.4 ± 2.1†</td>
<td>17.8 ± 2.1§</td>
</tr>
<tr>
<td>FA, impulses/s</td>
<td>Base</td>
<td>0.30 ± 0.06</td>
<td>0.30 ± 0.10</td>
<td>0.45 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>Peak</td>
<td>5.12 ± 0.81§</td>
<td>4.34 ± 0.66§</td>
<td>3.94 ± 0.58§</td>
</tr>
</tbody>
</table>

Values are means ± SE. Effect of intravenous capsaicin, bradykinin, and substance P challenge on heart rate (HR), arterial blood pressure (ABP), pulmonary insufflation pressure (PIP), and C-fiber activity (FA) in anesthetized guinea pigs (n = 8, 1 fiber/animal). *P < 0.05, †P < 0.01, §P < 0.005, ¶P < 0.001 vs. baseline (Base).
tested that SP activates C fibers through arachidonic acid metabolites.

In these series of experiments, C fibers were challenged with capsaicin and SP before and after administration of indomethacin (1 mg/kg). Indomethacin administration did not attenuate C-fiber activation or the PIP increase induced by capsaicin challenge (Fig. 6). Mean baseline and peak C-fiber activity and PIP are shown in Fig. 8, A and B. In response to capsaicin challenge, the increase in C-fiber activity before indomethacin administration was 6.2 ± 1.3 before and was 5.9 ± 1.0 impulses/sec after indomethacin administration (P > 0.05). The increase in PIP was 23.6 ± 5.5 before and 34.0 ± 4.0 cmH2O after indomethacin administration (P = 0.03).

C-fiber activation by SP was attenuated by indomethacin administration (Fig. 7). Mean baseline and peak C-fiber activity and PIP are shown in Fig. 8, C and D. In response to SP challenge, the increase in C-fiber activity before indomethacin administration was 4.6 ± 0.8 compared with 1.0 ± 0.3 impulses/s after indomethacin administration (P = 0.0002). The change in PIP was 15.9 ± 4.4 before and 9.0 ± 2.8 cmH2O after indomethacin administration (P = 0.10).

The effects of prostaglandin challenge of C fibers in guinea pigs have not been reported in the literature. In the present study, C fibers were challenged with intravenous injections of prostaglandin E2 and F2α. Both prostaglandin E2 and F2α activated C fibers (Fig. 9, A and B). These data, along with that of the other variables monitored during the experiment, are presented in Table 2. The profile of C-fiber activation and PIP after PGE2 and PGF2α challenges is presented in Fig. 10.

**DISCUSSION**

The present study demonstrates that exogenously applied SP stimulates an indomethacin-sensitive pathway that indirectly leads to C-fiber activation. An in vivo extracellular recording technique was utilized in this study. It is a common experimental approach to studying airway afferent fiber activity. This approach offers the advantage of studying reflex outputs along
with afferent activity while the nerves are in their normal physiological environment.

This is the first study to report that SP activates lung C fibers. Lung C fibers produce and release neuropeptides such as SP (28). If the activation were direct, then this would be a feedforward mechanism. Such a mechanism is not unreasonable in hyperresponsive airways such as in asthma or in guinea pigs in which the airways are hyperresponsive to numerous endogenous agents including neuropeptides. Neuropeptide receptors have been identified on pulmonary and bronchial C fibers in the lungs (11). However, neuropeptide receptors are also present on epithelial cells (30), mast cells (26), macrophages (9), and eosinophils (16). Activation of these receptors may then release secondary mediators causing lung C-fiber stimulation.

To determine whether C-fiber activation by SP is direct or involves release of other endogenous mediators, C fibers were challenged with SP before and after either BK receptor blockade or cyclooxygenase (COX) inhibition. COX inhibition, but not BK receptor blockade, attenuated C-fiber activation by SP. A question arises as to whether the effect of indomethacin could be mimicked by selective COX-1 or COX-2 inhibitors.

Table 2. Effect of prostaglandin challenge

<table>
<thead>
<tr>
<th>Agent</th>
<th>HR, beats/min</th>
<th>ABP, mmHg</th>
<th>PIP, cmH₂O</th>
<th>FA, impulses/s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base</td>
<td>Peak</td>
<td>Base</td>
<td>Peak</td>
</tr>
<tr>
<td>10 μg PGE₂</td>
<td>193±7</td>
<td>195±8</td>
<td>47/27±3/3</td>
<td>39/16*±2/1</td>
</tr>
<tr>
<td>20 μg PGF₂α</td>
<td>191±6</td>
<td>190±8</td>
<td>48/25±3/3</td>
<td>50/25±5/4</td>
</tr>
</tbody>
</table>

Values are means ± SE. Effect of prostaglandin challenge on HR, ABP (systolic/diastolic pressure), PIP, and FA in anesthetized guinea pigs (n = 10, C fibers in 4 guinea pigs). *P < 0.005 vs. Base.
In guinea pig isolated perfused lung, COX-1 but not COX-2 inhibitors mimic the effect of indomethacin on inhibiting prostaglandin release (1).

Prostaglandin challenges of lung C fibers have been reported in dogs (12, 27). In rats, prostaglandin administration sensitizes C fibers to mechanical stimuli and certain chemical mediators such as capsaicin (18, 24, 25). Although PGF2α activates rapidly adapting receptors in guinea pigs (6), the effects of prostaglandins on C fibers have not been reported. In the present study, both PGE2 and PGF2α activated lung C fibers. This activation appeared largely independent of changes in lung mechanics and therefore appears to be direct. Taken together, these data suggest that SP activation of lung C fibers includes release of prostaglandins.

Earlier studies have shown that SP causes the release of PGE2 from isolated rat intrapulmonary bronchi (8, 31). This PGE2 release was attenuated with administration of either a NK1 antagonist or meclofenamate, suggesting that the action of SP in the present study is mediated by neurokinin type 1 receptors. Removal of the epithelium in these preparations also blocked SP-induced PGE2 release (8, 31). This suggests the source of C-fiber activation by PGE2 and PGF2α is the airway epithelium adjacent to C fibers in the airway walls.

Another possible action of prostaglandins is to induce the release of other mediators that activate C fibers. Although prior administration of indomethacin decreased C-fiber reactivity to SP, peak C-fiber activity was still elevated above baseline. C-fiber activation may also occur though mediators of the lipoygenase pathway of arachidonic acid metabolism. Hwang et al. (20) determined that products of lipoygenases, including leukotriene B4, activate cloned capsaicin receptors (VR1) in human embryonic kidney cells, whereas prostaglandins failed to activate VR1 receptors. Therefore, products of COX and lipoygenase may act through separate pathways to activate C fibers.

After indomethacin treatment, SP activation of some C fibers were blocked while others were attenuated. There are several possible explanations for this observation. The remain-

Fig. 10. Profile of FA (■) and PIP (▲) in 10-s bins vs. time after intravenous challenges of prostaglandin E2 (10 μg; A) and F2α (20 μg; B) and n = 10 C fibers in 4 guinea pigs.

Fig. 8. Capsaicin (A and C) and SP challenges (B and D) of FA and PIP, respectively, before and after indomethacin administration (1 mg/kg iv). Both agonist challenges were 1 μg iv and n = 8 guinea pigs with 1 fiber/animal. The arrow separates the response before and after antagonist administration. *P < 0.01 vs. base. +P < 0.01 comparison of variable change from baseline of before vs. change after BK receptor blockade.
ing response may have been because the dose of indomethacin chosen was insufficient to abolish prostanoid synthesis in the lungs, the COX-1 isoenzyme in particular. The dose of indomethacin used in this study has been shown to block bronchoconstriction by COX products in vivo in guinea pigs (17); however, a dose-response curve of indomethacin was not tested in this study.

Capsaicin was used to identify lung C fibers in the present study. Capsaicin has long been used to identify C fibers (13, 14, 19). The action of capsaicin has been assumed to be direct. In rats, capsaicin increases C-fiber activity without increasing insufflation pressure (7). Capsaicin activates C fibers through the VR1. The VR1 was the first cloned membrane-bound receptor for capsaicin (10). However, to further investigate the mechanism of C-fiber activation by capsaicin, capsaicin challenges were also performed both before and after either BK receptor blockade or COX inhibition. Neither BK receptor blockade nor COX inhibition attenuated C-fiber activation by capsaicin. This suggests that, in this case, the endogenous SP released by C-fiber stimulation after capsaicin challenge is not sufficient to activate these afferent fibers and that the dose of SP administered in this study is substantially greater than that released endogenously.

BK is one of the few endogenous agonists that consistently lead to action potential discharge when applied directly to the receptive fields of guinea pig airway afferent neurons (15, 21). BK B2 receptor stimulation depolarizes the membrane potential of nodose ganglion neuron cell bodies and inhibits the calcium-dependent potassium current that is responsible for an after-spike hyperpolarization (33). However, the latter effect on the after-spike hyperpolarization appears to be secondary to prostacyclin production by the neuron. The BK antagonist cocktail effectively antagonized the effect of BK on both C-fiber activation and increasing PIP.

In conclusion, the results demonstrate that exogenously applied SP activates C fibers through the release of prostanoid, at least in part. Possible direct C-fiber stimulation by SP is yet to be verified. Although both PGE2 and PGF2α mimicked the effect of SP, questions remain as to whether prostanoids or thromboxane are more relevant in this regard or if selective
prostaglandin receptor antagonists could mimic the inhibitory effect of indomethacin.

ACKNOWLEDGMENTS

Expert statistical consultation was provided by Dr. Gleb Haynatzki of the Dept. of Medicine, Creighton University.

GRANTS

This study was funded by a grant from the State of Nebraska Department of Health and Human Services Cancer and Smoking Program.

REFERENCES