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Evan's blue dye blocks capsaicin-induced cough and bronchospasm in the guinea pig

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Abstract

The influence of Evan's blue dye on capsaicin-induced bronchoconstrictor and cough responses was investigated in the guinea pig. Evan's blue (30 mg kg^{-1} i.v.) pretreatment shifted the bronchoconstrictor dose-response to capsaicin (0.3–100 $\mu g\ kg^{-1}$ i.v.) to the right by 10-fold, but had no effect on the bronchospasm elicited by neurokinin A (0.3–10 $\mu g\ kg^{-1}$ i.v.). Evan's blue (0.3–30 mg kg^{-1} s.c.) also inhibited capsaicin-induced cough in a dose-dependent manner. Evan's blue blocked capsaicin responses by the intravenous, subcutaneous, or inhaled routes of administration. We conclude that Evan's blue inhibits capsaicin-induced bronchoconstrictor responses and cough in vivo.

Keywords: Evan's blue; Capsaicin; Pulmonary response

Evan's blue (6,6'-[C3,3'-dimethyl[1,1'-biphenyl-4,4'-diyl)bis(azo)bis[4-amino-5-hydroxy-1,3-naphthalenedisulfonic acid] tetrasodium salt) is a water soluble dye that binds to plasma macromolecules in vivo (LeVeen and Fishman, 1947) and has been used to quantify experimentally induced plasma exudation in pulmonary tissues (Danko et al., 1992; Lundberg and Saria, 1982). As part of other studies, we unexpectedly observed a decrease in responsiveness to capsaicin in animals pretreated with Evan's blue. The purpose of this study was to further investigate the influence of Evan's blue on capsaicin-induced responses in several in vivo models.

Studies were performed on Dunkin-Hartley guinea pigs (250–600 g). The influence of Evan's blue was assessed on capsaicin or neurokinin-A-induced bronchospasm in anesthetized guinea pigs and capsaicin-induced cough in awake animals. The methods for both techniques have been described (Hey et al., 1993; Bolser et al., 1994). Briefly, guinea pigs were anesthetized with dialurethane (1.0 ml kg^{-1} i.p.), artificially ventilated and paralyzed with gallamine triethiodide (2.0 mg kg^{-1} i.v.). Pulmonary insufflation pressure was measured from a side-arm of the tracheal cannula and used as an index of bronchospasm (Hey et al., 1993). Evan's blue (30 mg kg^{-1} i.v.) or saline was administered 5 min before capsaicin or neurokinin A. Each animal served as its own control. To elicit coughing, unanesthetized guinea pigs were individually exposed to aerosols of capsaicin (300 μM) for 4 min. Coughs were detected by a microphone placed in the exposure chamber. Each animal was exposed only once to capsaicin. The influence of Evan's blue on capsaicin-induced cough was expressed as percentage inhibition of the average number of coughs observed in vehicle-treated control groups (Bolser et al., 1994). Evan's blue was administered either subcutaneously 30 min before capsaicin or by aerosol for 4 min before capsaicin. Data are expressed as mean ± S.E.M. $P < 0.05$ was considered significant. ED_{50} values are expressed as 50% of the maximum response.

Capsaicin (0.3–100 $\mu g\ kg^{-1}$ i.v.) increased pulmonary insufflation pressure in a dose-dependent manner (Fig. 1A). The maximum effect of capsaicin was an increase in pulmonary insufflation pressure approximately 45 cm H_2O over baseline. Pretreatment with Evan's blue shifted the dose-response relationship to the right by 10-fold ($ED_{50} = 3.2\ \mu g\ kg^{-1}$ for capsaicin; $ED_{50} = 31\ \mu g\ kg^{-1}$ for Evan's blue plus capsaicin) without altering the maximum bronchoconstrictor ef-

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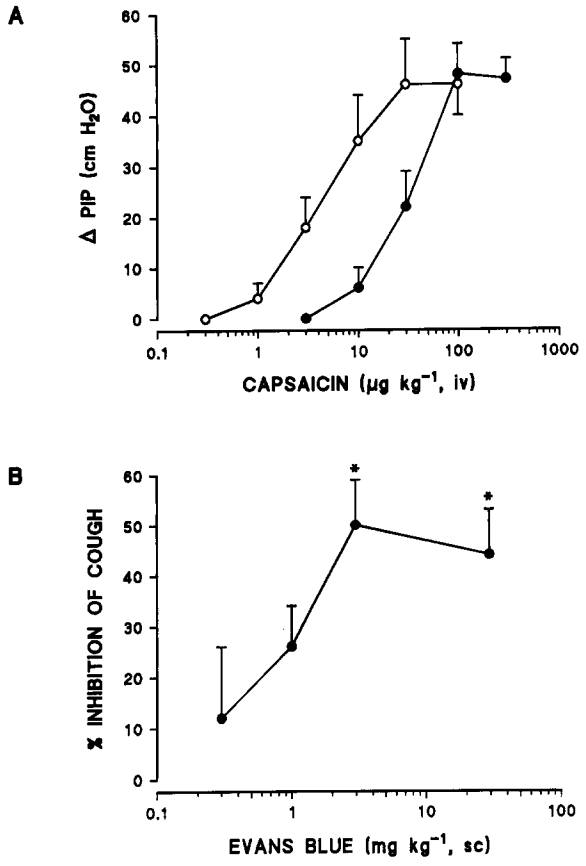

Fig. 1. Influence of Evan's blue on capsaicin-induced bronchospasm and cough. In panel A, the increase in pulmonary insufflation pressure (Δ pulmonary insufflation pressure) over baseline induced by capsaicin in the absence (○) and presence (●) of Evan's blue (30 mg kg^{-1} i.v.) is shown. Evan's blue pretreatment produced a rightward shift in the capsaicin dose response of 10-fold. In panel B, systemic administration of Evan's blue inhibits capsaicin-induced cough in a dose-dependent manner ($^{*}P < 0.05$ relative to control animals).

fect of capsaicin. Neurokinin A (0.3–10 μg kg^{-1} i.v.) increased pulmonary insufflation pressure in a similar dose-dependent manner (data not shown). Pretreatment with Evan's blue had no effect on the increase in pulmonary insufflation pressure after neurokinin A (ED_{50} = 2.0 μg kg^{-1} for neurokinin A; ED_{50} = 2.0 μg kg^{-1} for Evan's blue plus neurokinin A, $n = 5$ per group). Evan's blue administration had no effect on pulmonary insufflation pressure on its own.

Capsaicin-induced cough was inhibited by systemic pretreatment with Evan's blue (0.3–30 mg kg^{-1} s.c., $n = 12$ per dose) in a dose-dependent manner (Fig. 1B). Likewise, aerosol treatment with Evan's blue (1–300 μM) dose-dependently inhibited capsaicin-induced cough (data not shown, ED_{50} = 8 μM, $n = 12$ per dose). Evan's blue was devoid of nonspecific effects. Systemic treatment (s.c.) with Evan's blue did not elicit gross toxicity or depression of ventilation at doses as high as 300 mg kg^{-1} (3 ± 8% inhibition of ventilation at this dose, $n = 6$).

This is the first report showing that capsaicin responses are blocked by Evan's blue. Both the in vivo bronchoconstriction and cough elicited by capsaicin were inhibited by pretreatment with Evan's blue. The effects of Evan's blue were dose-dependent and specific to capsaicin. Evan's blue blocked capsaicin-induced responses by the intravenous, subcutaneous, or inhaled routes of administration.

Another dye, ruthenium red, acts as a capsaicin antagonist in other systems (Franco-Cereceda and Lundberg, 1989) as well as in cough induced by this irritant (Bolser et al., 1991). The mechanism of action of ruthenium red is thought to be blockade of capsaicin activated calcium channels (Dray et al., 1990). Whether or not Evan's blue and ruthenium red share a common mechanism or Evan's blue is a competitive antagonist of capsaicin receptors remains to be elucidated.

These results indicate that caution should be used when Evan's blue is used in conjunction with capsaicin. Pretreatment with Evan's blue will significantly attenuate the effects of capsaicin, requiring much higher doses of this irritant to elicit the desired response. Evan's blue is most commonly used as an indicator of plasma extravasation (Danko et al., 1992). Although we did not measure capsaicin-induced plasma extravasation in the presence and absence of Evan's blue, our results are most likely applicable to this measurement as well, because Evan's blue inhibited several different capsaicin responses by a variety of routes of administration.

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