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Delayed anti-inflammatory action of nedocromil sodium in the rat paw is dependent on de novo protein synthesis

Johan Raud *, David Konrad, Sven-Erik Dahlén

Department of Physiology and Pharmacology, and Institute of Environmental Medicine, Karolinska Institutet, S-171 77 Stockholm, Sweden

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Abstract

Nedocromil sodium is commonly suggested to reduce allergic inflammation by inhibiting mediator release from mast cells. However, nedocromil also exhibits a wide range of additional anti-inflammatory activities, including inhibition of increased vascular permeability induced by individual mediators such as histamine. In the present study, we have further characterized the mode of action of nedocromil in a rat model for hind paw edema. Mast cell-dependent edema was induced with compound 48/80 (edema response mainly due to 5-hydroxytryptamine release), and direct mediator-induced plasma extravasation was evoked by exogenous 5-hydroxytryptamine (both agents injected locally). Local pretreatment with nedocromil for 20 min dose-dependently inhibited the edema evoked by compound 48/80 more effectively than that induced by 5-hydroxytryptamine. However, after 2 h pretreatment, both the 5-hydroxytryptamine-and compound 48/80-induced edema responses were inhibited to approximately the same extent by a range of concentrations of nedocromil, as well as by dexamethasone. Local inhibition of RNA/protein synthesis with actinomycin-D abolished the effects of both dexamethasone and nedocromil (2 h local pretreatment). We thus conclude that nedocromil can produce an 'anti-exudative' effect that is independent of inhibition of mast cell mediator release, is slow in onset, and requires de novo protein synthesis.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); Actinomycin-D; Dexamethasone; Mast cell; Nedocromil sodium; Paw edema, rat

1. Introduction

Nedocromil sodium is a compound with chemical and biological properties similar to those of sodium cromoglycate, and it is used in the treatment of asthma and allergic rhinitis and conjunctivitis (Thomson, 1989; Ciprandi et al., 1992; Brogden and Sorkin, 1993; Parish and Miller, 1993). Both nedocromil and cromoglycate are classically believed to reduce allergic inflammation by inhibiting mediator release from mast cells (Wells et al., 1986; Riley et al., 1987; Leung et al., 1988). However, more recent studies indicate that nedocromil also possesses anti-inflammatory properties unrelated to inhibition of mast cell activation. Thus, nedocromil has been shown to inhibit pro-inflammatory effector functions of eosinophils, neutrophils, macrophages, platelets, epithelial cells, as well as sensory nerves (Rainey, 1989; Thomson, 1989; Brogden and Sorkin, 1993). Moreover, topically applied nedocromil inhibits increased vascular permeability induced by exogenous histamine (Dahlén et al., 1989). Because mediators such as histamine and 5-hydroxytryptamine (5-HT) are known to cause edema by contracting endothelial cells (Majno and Palade, 1961; Thureson-Klein et al., 1987; Wu and Baldwin, 1992; Svensjö, 1990), the observed inhibition of increased vascular permeability indicates that nedocromil also inhibits endothelial cell function. The detailed mechanism(s) by which nedocromil exerts this wide range of anti-inflammatory actions is not clear. Most likely, nedocromil interferes with some common step(s) in the cascade of events associated with stimulus-response coupling in inflammatory cells.

Another group of compounds with a very broad spectrum of anti-inflammatory activities are the glucocorticoids (Schleimer et al., 1989). It is well established

* Corresponding author. Department of Physiology and Pharmacology, Karolinska Institutet, S-171 77 Stockholm, Sweden. Tel. +46 8-325990, fax +46 8-332047.

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that the expression of anti-inflammatory activity by steroids requires protein (e.g. lipocortin) synthesis and is therefore slow in onset with a time lag of 1 h or more (Tsurufuji and Ohuchi, 1989; Flower, 1989). Interestingly, there are data to indicate that nedocromil and glucocorticoids exhibit similar activities with respect to inhibition of plasma extravasation induced by histamine (Björk et al., 1985; Dahlén et al., 1989; Svensjö, 1990). Prompted by these observations, we now report on a study designed to examine whether or not nedocromil displays glucocorticoid-like activity, i.e. an inhibitory effect on mediator-induced increased vascular permeability that is slow in onset and requires protein synthesis. Using a model for rat paw edema, we compared time-and dose-dependent effects of nedocromil on edema responses evoked by 5-HT and the mast cell secretagogue compound 48/80. Moreover, we examined the effects of nedocromil and dexamethasone on 5-HT-induced paw swelling in the presence and absence of actinomycin-D, a commonly used inhibitor of protein synthesis at the level of transcription. We found that nedocromil indeed exerts a delayed anti-inflammatory effect of which a major part appears to be mediated via induction of protein synthesis.

2. Materials and methods

Hind paw edema was induced in male Sprague-Dawley rats (160–180 g) during light ether anesthesia by subplantar injection of 5-HT (Sigma Chemical Co., St. Louis, MO, USA; 10 nmol in 50 μl sterile phosphate-buffered saline (PBS)) into one paw and compound 48/80 (Sigma; 5 μg in 50 μl PBS) into the other, or 5-HT into both paws. One or both paws of each rat were pretreated locally by injecting various doses (0.003–3 μmol in 100 μl PBS) of nedocromil sodium (Fison Pharmaceuticals, Leicestershire, UK) 20 min or 2 h prior to challenge with 5-HT or compound 48/80. Depending on the experimental design (see Results) the effects of nedocromil were compared with the contralateral paw exposed to vehicle and agonist, or with a parallel group of control animals with both paws pretreated with vehicle prior to agonist.

The influence of inhibition of protein synthesis on the anti-inflammatory effects of nedocromil (0.03 μmol, 2 h pretreatment) or dexamethasone (Decadron, MSD, Rahway, NJ, USA; 1 μg, 2 h pretreatment) was tested using actinomycin-D (Sigma). These experiments were performed by injecting rats with either dexamethasone or nedocromil into one paw, and a mixture of actinomycin-D (2.5 μg dissolved in PBS) and the respective drug into the other paw. The effects of actinomycin-D were examined by comparing the two paws of each rat. Potential unspecific effects of actinomycin-D were first tested in separate experiments. One group of rats was pretreated with the 5-HT antagonist methysergide (Sandoz, Basel, Switzerland; 0.1 mg/kg i.p., total volume 1 ml) 30 min prior to injection of 5-HT into one paw and compound 48/80 into the other paw. This group was compared with control animals pretreated with 1 ml PBS i.p.

Paw volumes were measured using a hydroplethysmometer (Ugo Basile, Varese, Italy) before and 20 min or 2 h after start of treatment, as well as 30 min, 1 h, 2 h and 3 h after challenge with 5-HT or compound 48/80. Paw swelling was expressed either as percent increase in paw volume over time, or as total paw swelling by computer-assisted planimetry of areas under the time-curve (see e.g. Fig. 1) for paw swelling during 0–3 h.

Statistical analysis was performed using Student's paired or unpaired t-test. The results are presented as mean values ± S.E. The project was approved by the Regional Ethical Committee for Animal Experimentation.

3. Results

The doses of 5-HT (10 nmol) and the mast cell secretagogue compound 48/80 (5 μg) were chosen so as to cause quantitatively and temporally similar increases in paw swelling (Fig. 1). As compared to the values prior to stimulation, the paw volumes increased by approximately 50%, 30 min after challenge with 5-HT or compound 48/80 (from ≈ 1.20–1.50 ml to ≈ 1.80–2.20 ml), whereafter a gradual decrease oc-

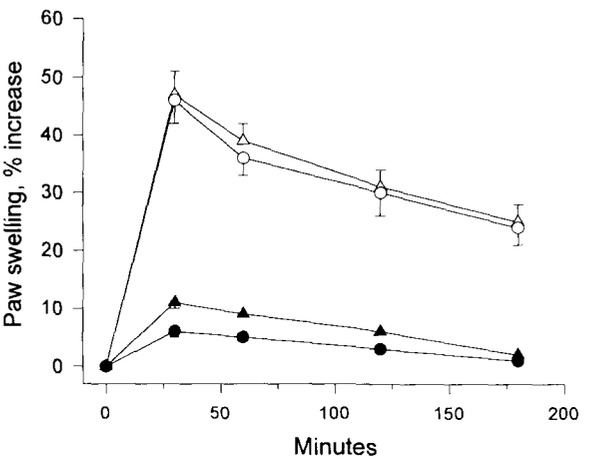

Fig. 1. Hind paw edema in rats induced by subplantar injection of 5-HT (10 nmol, open circles) into one paw and compound 48/80 (5 μg, open triangles) into the other paw in vehicle-treated animals. Closed symbols indicate pretreatment with the 5-HT antagonist methysergide (0.1 mg/kg i.p.) 30 min prior to challenge with 5-HT (circles) and compound 48/80 (triangles) (mean ± S.E., $n = 5$ in each group). The inhibition by methysergide is highly significant ($P < 0.001$) for both 5-HT and 48/80 at all time-points (not indicated in figure for clarity).

curred during the following 3 h. In line with previous observations (see Green et al., 1979), systemic pretreatment with the 5-HT antagonist methysergide revealed that the mast cell-dependent response to compound 48/80 was almost entirely mediated by endogenous 5-HT (Fig. 1), strongly suggesting that the edema responses to 5-HT and compound 48/80 were mediated via the same mechanism at the target organ (i.e. 5-HT acting on the endothelial cell).

Table 1 summarizes the effects of 20 min or 2 h pretreatment with different doses of nedocromil on total (0–3 h) paw edema induced by 5-HT and compound 48/80. As shown, pretreatment with nedocromil for 20 min caused a distinct but relatively small dose-dependent inhibition of the direct edema evoked by 5-HT. With the same nedocromil treatment, the response to compound 48/80 was reduced to a much greater extent. After 2 h pretreatment, on the other hand, both the 5-HT-and the compound 48/80-induced edema responses were equally inhibited by the range of doses of nedocromil. To further emphasize the differences in effect by varying the pretreatment time of nedocromil, 20 min pretreatment with 0.3 μmol nedocromil inhibited mainly the response to compound 48/80, whereas 2 h pretreatment with a 10-fold lower dose (0.03 μmol) of nedocromil reduced the responses to compound 48/80 and 5-HT to the same degree. These findings suggested that, with time, the anti-edema effect of nedocromil was relatively more important than the 'mast cell stabilizing' action of nedocromil.

The dose-response relationship for 2 h pretreatment with nedocromil tended to be bell-shaped (Table 1). It was therefore important to examine if the higher doses of nedocromil were pro-inflammatory in themselves. However, nedocromil per se did not cause edema formation, i.e. as assessed 20 min or 2 h after administration of 0.3 or 3 μmol nedocromil ($n = 24$), the mean increase in paw volume was < 3% and not significantly different from injection of vehicle (the same applies to the lower doses of nedocromil).

To examine whether or not nedocromil acted via

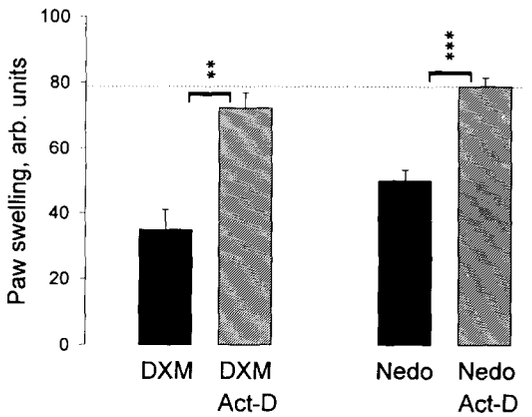

Fig. 2. Effects of inhibition of protein synthesis with actinomycin-D on anti-edema effects of dexamethasone or nedocromil on 5-HT-induced rat hind paw edema. The experiments were performed by injecting rats with either dexamethasone (DXM, 1 μg, $n = 6$) or nedocromil (Nedo, 0.03 μmol, $n = 12$) into one paw (filled bars), and a mixture of actinomycin-D (Act-D, 2.5 μg) and the respective drug into the other paw (hatched bars) 2 h prior to local challenge with 5-HT (10 nmol). The effects of actinomycin-D were examined by comparing the two paws of each rat, and expressed in arbitrary units for total paw swelling during 0–3 h (see Materials and methods for experimental details). The dashed line indicates the control response to 5-HT in a separate group of vehicle-treated rats (relative value, $n = 6$). ** $P < 0.01$, *** $P < 0.001$ (mean ± S.E.).

synthesis of messenger proteins, experiments were carried out where nedocromil and dexamethasone pretreatment (2 h) was performed in the presence of actinomycin-D. As expected, when actinomycin-D (2.5 μg per paw) was mixed with dexamethasone (1 μg per paw), the anti-inflammatory effect of dexamethasone on 5-HT-induced edema was reduced considerably (Fig. 2). Interestingly, actinomycin-D inhibited the corresponding effect of nedocromil (0.03 μmol) in a very similar fashion (Fig. 2). The paw swelling in response to 5-HT in the presence of nedocromil + actinomycin-D and dexamethasone + actinomycin-D was 94 ± 12% and 100 ± 17%, respectively, of the control response to 5-HT in a separate group of vehicle treated rats ($n = 6$), i.e. actinomycin completely reversed the anti-edema effects of both dexamethasone and nedocromil. The

Table 1
Inhibition (%) of rat paw edema by nedocromil sodium

	Agonist	Nedocromil (μmol per paw)			
		0.003	0.03	0.3	3
20 min	5-HT	n.d.	6.4 ± 2.0	13.1 ± 2.9 **	25.7 ± 8.3 *
	48/80	n.d.	18.7 ± 4.8 *	40.1 ± 3.8 ***	37.8 ± 6.1 **
2 h	5-HT	13.0 ± 7.5	36.3 ± 3.8 ***	28.5 ± 11.1 *	22.8 ± 2.6 ***
	48/80	19.8 ± 5.9	32.3 ± 4.8 ***	23.9 ± 10.7 *	20.1 ± 1.8 ***

Hind paw edema in rats induced by subplantar injection of 5-HT (10 nmol) into one paw and compound 48/80 (5 μg) into the other. Both paws of each rat pretreated locally by injecting different doses of nedocromil 20 min or 2 h prior to agonist challenge. Values represent percent inhibition of agonist-induced total paw swelling during 0–3 h (see Materials and methods for details). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (mean ± S.E., $n = 4$–9). n.d. = not done.

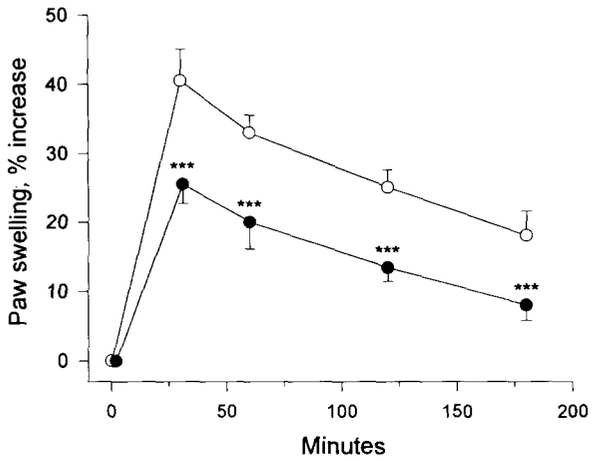

Fig. 3. Hind paw edema in rats induced by bilateral subplantar injection of 5-HT. One paw of each rat was pretreated with vehicle (open circles) and the other paw with nedocromil (0.03 μmol, closed circles) 2 h prior to challenge with 5-HT (10 nmol). *** $P < 0.001$ (mean ± S.E., $n = 5$ in each group).

dose of actinomycin-D used in the experiments was selected by first confirming that the drug by itself neither caused paw edema nor suppressed or enhanced the responses induced by 5-HT (not shown). Moreover, two full series of blocking experiments with actinomycin-D were performed, using two different batches of actinomycin-D. The results with respect to reversal of effects of nedocromil and dexamethasone were almost identical at these two occasions (data combined in Fig. 2).

Finally, experiments were performed to exclude that nedocromil acted via some non-specific systemic route, e.g. by causing release of endogenous corticosteroids. Thus, nedocromil was injected into one paw and PBS into the other, and after 2 h both paws were challenged with 5-HT. In these paired experiments it was found that the anti-edema action of nedocromil was restricted to the site of drug injection (Fig. 3, compare with Fig. 1).

4. Discussion

We found that nedocromil sodium expressed two distinct modes of anti-inflammatory action in a rat model for 5-HT-induced and mast cell-dependent (compound 48/80) hind paw edema. Thus, 20 min local pretreatment with nedocromil predominantly inhibited the edema evoked by compound 48/80, which was shown to act mainly via release of endogenous 5-HT, whereas 2 h pretreatment with nedocromil inhibited the edema evoked by compound 48/80 and exogenous 5-HT to an equal extent. Knowing that 5-HT increases vascular permeability by causing interendothelial gaps (Majno and Palade, 1961), the findings suggest that the delayed anti-edema action of nedocromil was an effect on the barrier function of the microvascular endothelium, and that, with time, the action of nedocromil on mast cell mediator release became comparatively less important. A gradually developing inhibition of direct mediator-induced increased vascular permeability is a characteristic of glucocorticoids, which act by inducing synthesis of anti-inflammatory proteins (Flower, 1989; Tsurufuji and Ohuchi, 1989). In this study, we were able to show that nedocromil also appears to express a slowly evolving anti-inflammatory action related to induction of protein synthesis. Thus, the delayed anti-edema effect of both dexamethasone and nedocromil was abolished by actinomycin-D, one of the most commonly used inhibitors of glucocorticoid actions that require protein synthesis. The action of actinomycin-D appeared specific because it neither caused paw edema by it self, nor did actinomycin-D suppress or enhance the response to injection of 5-HT alone. It is also worth noting that 'anti-glucocorticoids' such as actinomycin-D do not interfere with the action of anti-inflammatory proteins already synthesized by cells exposed to glucocorticoids (Tsurufuji and Ohuchi, 1989). It may be argued that nedocromil treatment somehow caused release of endogenous corticosteroids and that such a mechanism could explain the delayed anti-edema effect of nedocromil. However, this possibility was excluded in separate experiments which showed that the anti-edema action by nedocromil was strictly local.

To the best of our knowledge, delayed protein synthesis-dependent anti-inflammatory actions by nedocromil have not been described previously. It will be of interest to evaluate if our findings have bearing on other in vitro and in vivo systems where nedocromil expresses anti-inflammatory actions. A potentially related mode of action of nedocromil has been indicated in experiments with human bronchial epithelial cells where nedocromil inhibited interleukin-1-induced production of interleukin-8 with a time lag of several hours (Vittori et al., 1992).

We noted that the dose-response relationship for 2 h pretreatment with nedocromil tended to be bell shaped. One possible explanation for this observation would be that higher doses of nedocromil are pro-inflammatory in themselves. However, injection of nedocromil per se did not cause detectable edema or erythema as assessed 20 and 2 h after administration, supporting that nedocromil per se had no pro-inflammatory activity. The latter observation also suggests that the anti-edema action by nedocromil was specific and not the result of some unspecific 'counter-irritant' effect. The cause of the bell-shaped dose-response relationship for the anti-exudative effect of nedocromil remains unknown; however, it is worth noting that the pharmacology of nedocromil has been

reported to be similar in other experimental systems as well (Riley et al., 1987).

In conclusion, our novel findings suggest that nedocromil sodium can produce an 'anti-exudative' effect that is slow in onset, requires de novo protein synthesis, and is independent of inhibition of mast cell mediator release. Additional mechanistic studies in different experimental or clinical models are needed to determine the relative importance of this steroid-like action of nedocromil, and may possibly reveal new approaches for drug development.

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