Discussion

Carbamazepine exerts a more potent inhibitory effect on the epileptiform activity induced by penicillin in the CA3 area than on that induced by $BaCl_2$. We showed that the effect of carbamazepine on penicillin-induced epileptiform discharges could be diminished by adding $BaCl_2$ to the perfusion medium. During exposure to $BaCl_2$, spontaneous epileptiform discharges induced by penicillin were moderately attenuated, indicating that the driving force of this activity may have been inhibited. The cause of this unexpected effect remains to be elucidated. Given the reduced intensity of epileptiform activity during the combined application of penicillin and $BaCl_2$, carbamazepine might have been expected to suppress these discharges more easily. On the contrary, however, its depressant effect was reduced, indicating that $BaCl_2$ antagonized the action of carbamazepine. Although these findings lend support to the potassium-flux hypothesis, they are not conclusive: Ba^{2+} ions penetrate the Ca^{2+} channels. For that reason we subsequently used the relatively selective potassium-channel blocker 4-AP. This agent strongly antagonized the action of carbamazepine and at the same time only transiently increased the epileptiform activity induced by penicillin. The inhibition of the action of carbamazepine by 4-AP is therefore unlikely to have been the result of changes in spontaneous epileptiform activity in the CA3 area. Moreover, carbamazepine did not diminish the epileptiform discharges induced by 4-AP alone.

In most of the experiments, rather high concentrations of carbamazepine were chosen. The fact that high concentrations of carbamazepine were strongly antagonized by 4-AP suggests that this is a potent blocker of the effects of carbamazepine. In patients receiving chronic treatment with carbamazepine the cerebrospinal fluid concentrations were reported to range from 4 to 14 μM[9].

It has been suggested that the anticonvulsant properties of carbamazepine could be due to its action on sodium channels[10]. Previous studies in 'epileptic' hippocampal slices demonstrated that carbamazepine has a postsynaptic depressant effect, and that it does not facilitate GABA-mediated paired-pulse inhibition[4,11]. Our present findings are not in conflict with these observations. An effect on the potassium channels tending to increase potassium fluxes would act in the same direction, namely to reduce cell excitability, and, perhaps even more specifically, to diminish repetitive firing.

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Aspirin-like drugs may block pain independently of prostaglandin synthesis inhibition

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Summary. Using flurbiprofen, a chiral anti-inflammatory and analgesic 2-arylpropionic acid derivative, the enantiomers of which are not converted to each other (less than 5%) in rats or man, we obtained evidence that prostaglandin synthesis inhibition is primarily mediating the anti-inflammatory activity but prostaglandin synthesis independent mechanisms contribute to the analgesic effects. Thus, the S-form inhibited prostaglandin synthesis, inflammation and nociception in rats. The R-form had much less effect on prostaglandin synthesis and did not affect inflammation. It did, however, block nociception in rats almost as potently as the S-form. S-flurbiprofen, in contrast to the R-form, was clearly ulcerogenic in the gastrointestinal mucosa. These results indicate additional molecular mechanisms of analgesia and suggest the use of R-arylpropionic acids as analgesics.
Key words. Aspirin-like drugs; flurbiprofen enantiomers; anti-inflammatory; analgesic; gastrointestinal toxicity; prostaglandin synthesis; rat.

Aspirin-like drugs are believed to exert their anti-inflammatory and analgesic activities via inhibition of the cyclo-oxygenase system, i.e., by reduction of pro-inflammatory and pro-analgesic prostaglandins, particularly in damaged tissue[1-5]. This contention, however, does not fully suffice the cause. There is no satisfying correlation between prostaglandin synthesis inhibition and analgesic effects[6]. Some non-acidic, non-opioid analgesics such as acetaminophen or phenazone do not inhibit prostaglandin production at analgesic concentrations[7,8], and the oldest compound of all, salicylic acid, does not inhibit cyclo-oxygenase at analgesic concentrations[7] or doses[9]. Recently, optically pure enantiomers of 2-arylpropionic acids have become available[10-12]. These compounds show a variable degree of conversion into each other in man and most laboratory animals[13,14]. The enantiomers of flurbiprofen, which can be analyzed stereoselectively in body compartments[15,16], are converted into each other to a negligible extent only in rats and man[10,13]. Using standard experimental models we determined the anti-inflammatory, anti-nociceptive (analgesic) and toxic effects together with the inhibition of prostaglandin production by S- and R-flurbiprofen in cells and tissues.

In figure 1 we show that R-flurbiprofen has only 1/500 the activity as compared to S-flurbiprofen as an inhibitor of prostaglandin production in macrophages. By contrast, R-flurbiprofen is almost as active as S-flurbiprofen as an analgesic in two different rat models of pain and

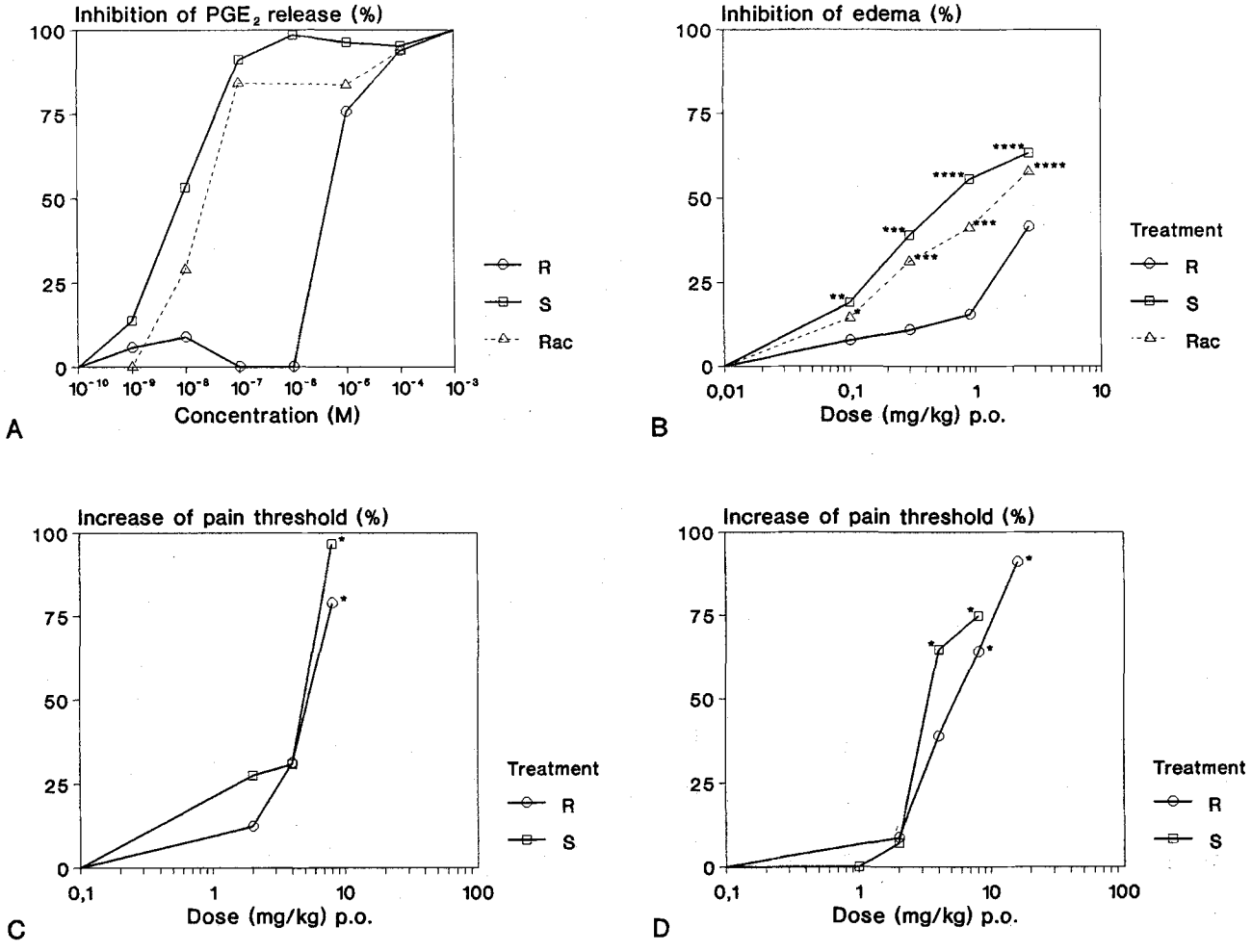

Figure 1. Pharmacodynamic effects of R- and S-flurbiprofen. Inhibition of prostaglandin production by mouse peritoneal macrophages (A) was determined as described previously[7]. S-flurbiprofen (S) (IC_{50} = 8.08 [-lg M]) was slightly more potent than racemic flurbiprofen (Rac) (IC_{50} = 7.62) or indomethacin (IC_{50} = 7.46, not shown) but more than 500 times more potent than R-flurbiprofen (R) (IC_{50} = 5.34). The anti-inflammatory effect (B) of S-, racemic and R-flurbiprofen as defined in the carrageenan edema of the rat paw[28] was related to their potency as PG-synthesis inhibitors in vitro. By contrast, the S- and R-enantiomers were of almost identical potency as antinociceptive drugs (C,D) in the rat Randall-Selitto assay[29] following the injection of interleukin-1 (IL-1) (C), method described in reference[30], or following the injection of baker's yeast (D) (comparison of the pain threshold of the inflamed paw between treated and control animals). Indomethacin used as a well-known reference substance was effective in all animal tests (3 mg/kg significant 50%-inhibition of carrageenan-induced edema, 6 mg/kg significant 50%-increase of pain threshold as well as both after IL-1- and yeast-induced pain). Inhibition and increase were calculated from the means of n = 5 (A) macrophage cultures, n = 6 (B,D) or n = 12 (C) male Sprague-Dawley rats per dose as compared to controls (n = 5, 6 or 12), respectively. Statistical analysis (B,C,D) by Student's 2-sided t-test for unpaired data. Values (edema or pain threshold, respectively) significant different as compared to control at $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ and $****p < 0.0001$.

nociception (fig. 1C and D). These results were obtained by blind observation which in turn was repeated twice with similar results. Moreover, both compounds showed similar analgesic activity in the mouse acetic writhing test (data not shown), another standard model of nociception. S-flurbiprofen was an effective ulcerogen in rats, damaging both the stomach of fasted animals as well as the small intestine of fed animals (fig. 2). R-flurbiprofen was devoid of both damaging effects at analgesic doses. An investigation of the PG synthesis inhibition in inflamed tissue (table), as well as the mucosa of the stomach and the small intestine shows (fig. 3) that, in line with current concepts, S-flurbiprofen inhibited prostaglandin production (PGE_2 and PGI_2) almost completely. R-flurbiprofen at identical doses had only marginal effects on prostaglandin production in gastric mucosa, and was devoid of any measurable effects in the mucosa of the small intestine. An analysis of the content of both enantiomers in plasma and the inflammatory fluid showed a) that both enantiomers had approximately equal access to these body compartments (table) and b) that there was very little conversion of R-flurbiprofen into S-flurbiprofen (2–5%).

Taken together, these results confirm that prostaglandin synthesis inhibition is of dominant importance as a mechanism of action of aspirin-like drugs with regard to

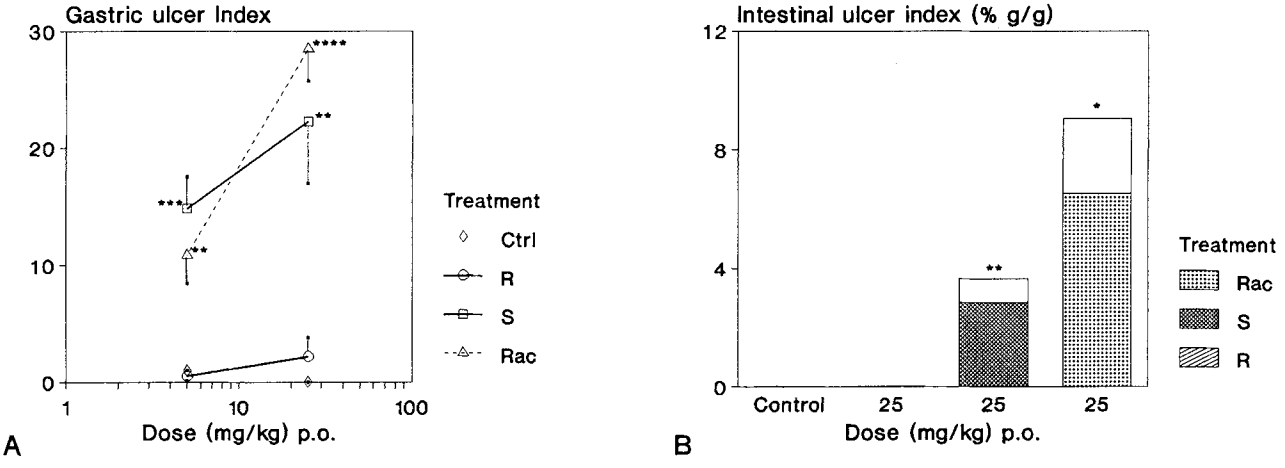

Figure 2. Gastrointestinal toxicity of R- and S-flurbiprofen in rats. Fasted (A) or fed (B) male rats were subjected to 5 or 25 mg/kg b.wt of R-, S- or racemic (Rac) flurbiprofen. Five (A) or 25 (B) hours later the rats were sacrificed and the ulcer index of the stomach (fasted rats) or of the small intestine (fed rats) was evaluated, respectively, as described previously[27, 31]. R-flurbiprofen did not cause significant mucosal damage in the GI-tract. Each point or column represents the mean +/− SEM of n = 6 (A) or n = 9 (B) male Sprague-Dawley rats. Statistical analysis by Student's 2-sided t-test for unpaired data. Values significantly different as compared to control (Ctrl) at *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$.

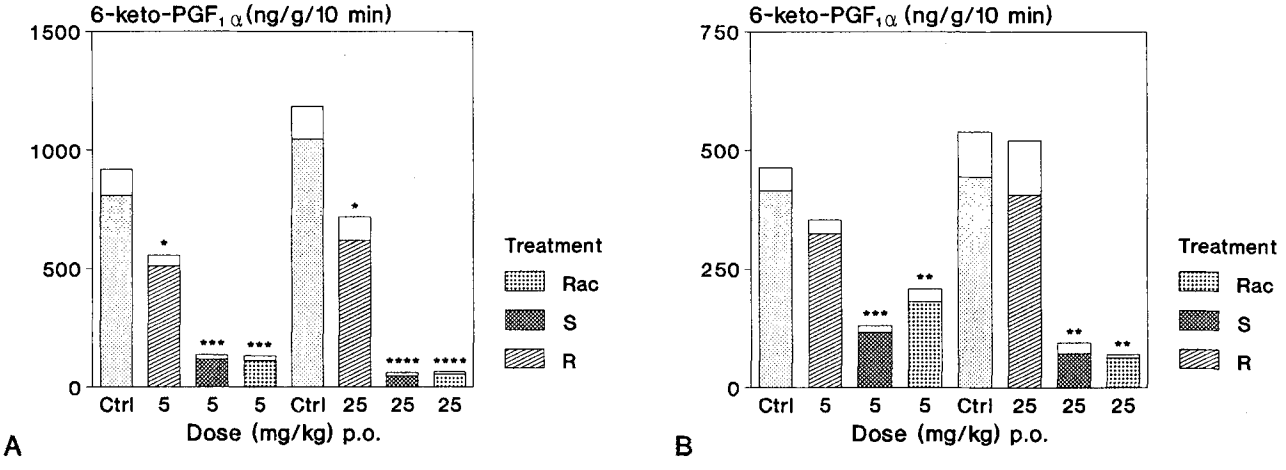

Figure 3. Effects of R- and S-flurbiprofen on prostaglandin (PG) release from specimens of the gastric (A) and jejunal (B) mucosa. Male Sprague-Dawley rats were treated as described in figure 2 (all animals were sacrificed 5 h after administration). Specimens of gastrointestinal mucosa were recovered and the capacity to release prostacyclin (measured as 6-keto-$PGF_{1\alpha}$) was defined following standard methods[27]. R-flurbiprofen was weakly effective (gastric mucosa) or inactive (jejunal mucosa) in contrast to S- or racemic (Rac) flurbiprofen. Each column represents mean + SEM of n = 6 animals. Statistical analysis by Student's 2-sided t-test for unpaired data. Values significantly different as compared to control (Ctrl) at *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$.

R- and S-flurbiprofen concentrations (amounts) and effects on prostaglandin production

Treatment Time	Plasma concentration R (μg/ml)	Plasma concentration S (μg/ml)	Amounts found in air pouches R (μg)	Amounts found in air pouches S (μg)	6-keto-$PGF_{1\alpha}$ (ng)	PGE_2 (ng)
Control						
2 h	ND	ND	ND	ND	4.69 ± 2.27	2.33 ± 1.01
6 h	ND	ND	ND	ND	106.63 ± 18.19	150.12 ± 31.76
R-flurbiprofen						
2 h	10.20 ± 1.16	0.65 ± 0.07	2.19 ± 0.70	0.06 ± 0.02	12.71 ± 5.98	9.91 ± 6.86
6 h	3.94 ± 0.93	0.50 ± 0.15 #	1.13 ± 0.19	0.07 ± 0.03 #	41.55 ± 15.49 *	62.58 ± 24.25
S-flurbiprofen						
2 h	ND	17.23 ± 1.65	ND	2.65 ± 1.22	<0.18 ± 0.01	<0.37 ± 0.03
6 h	ND	12.73 ± 2.35 #	ND	6.22 ± 0.85 #	<0.21 ± 0.01 ***	<0.41 ± 0.02 ***

Five mg/kg b.wt of S- or R-flurbiprofen were administered p.o. Carrageenan was injected simultaneously into the cavity of 6-day-old air pouches of male Sprague-Dawley rats according to standard methods [25, 26]. The animals were killed 2 and 6 h later, the air pouches rinsed and blood collected. Air pouch fluid was assayed for prostaglandin (PG) content as described [27]. R- and S-flurbiprofen were determined in air pouch fluid and plasma according to reference [16]. In the untreated and R-flurbiprofen-treated rats the amounts of 6-keto-$PGF_{1\alpha}$ and PGE_2 increased in the inflamed tissue whereas in the S-flurbiprofen-treated animals the contents of prostaglandins remained low. In accordance with our own pharmacokinetic studies (manuscript in preparation) and other reports [10, 13] the conversion of R-flurbiprofen into the S-enantiomer was negligibly small (2–5% in plasma). The 6-h values reflect the much slower elimination of S in comparison with R (#). Values represent means ± SEM of n = 6 or n = 5 animals. Statistical analysis of prostaglandins by Student's 2-sided t-test for unpaired data. Values significantly different as compared to control at * $p < 0.05$ and *** $p < 0.001$. ND = not done.

inflammation and GI-tract toxicity [1, 2, 5]. In addition, we present new evidence that these compounds interfere with nociception and pain through other mechanisms as well. The small amount of S-flurbiprofen produced from the R-enantiomer after administration of the latter may explain the anti-inflammatory effect of high doses but not the analgesic effects observed at low doses. These additional mechanisms of analgesia have not been clearly defined up to now. One may speculate that the stereoselective incorporation of R-enantiomers of 2-arylpropionic acids into lipids of cell membranes [17, 18] may interfere with the production of the second messenger diacylglycerol. By that, they may modulate the opening and closing of e.g., potassium channels via transduction pathways, involving calcium mobilization from intracellular storage pools and the activation of the protein kinase C enzyme family [19]. Consequently, the resting potential of nociceptive nerve endings may be enhanced. This assumption would demand that the antinociceptive effect of both enantiomers should vary according to the degree of incorporation into lipid membranes. Such data for flurbiprofen are lacking at present. Other explanations, e.g. a central site of action [20, 21] deserve, however, investigation as well. We attempted to obtain support for the latter contention by applying the evaluation procedure of the Randall-Selitto test suggested for detecting central analgesic effects. The results were inconsistent (data not given), in agreement with observations made by others [22]. On the other hand, pharmacokinetic studies of R- and S-flurbiprofen in rats showed a substantially higher peripheral volume of distribution ($V_{R-flurbiprofen}$ = 0.028 l ± 0.013 l, $V_{S-flurbiprofen}$ = 0.014 l ± 0.003 l; n = 3) of R-flurbiprofen compared to the S-enantiomer hinting at higher concentrations outside the plasma, i.e. in peripheral tissues as e.g. fat or brain. Both interpretations concerning the additional mechanisms of analgesia remain speculative at present.

Our results may also help to explain why some aspirin-like drugs are apparently better analgesics than others [6]. More importantly they indicate why salicylic acid is an analgesic as potent as aspirin [5, 23, 24], despite its lack of prostaglandin synthesis inhibition at analgesic doses. At such dosage regimens only salicylate from aspirin is likely to reach inflamed tissue [23]. According to our data we suggest that salicylate, as with the R-enantiomer of flurbiprofen, may be mediating its analgesic effects along alternative pathways at low doses. At high doses prostaglandin synthesis inhibition and anti-inflammatory effects become prominent.

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Effects of the pyrimidine-containing cytochrome P-450 inhibitor, fenarimol, on the formation of 20-OH ecdysone in flies

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Summary. The effect of fenarimol, a pyrimidine-containing cytochrome P-450 inhibitor, was tested in vitro on brain-ring gland complexes of *Calliphora vicina* (Dipt., Calliphoridae), and on microsomes prepared from the fat body of 0-h wandering stage larvae of *Neobellieria bullata* (Dipt., Sacrophagidae). Fenarimol had no influence on the formation of ecdysone, but it was an effective inhibitor of cytochrome P-450-dependent ecdysone 20-monooxygenase.
Key words. Calliphora vicina; *Neobellieria bullata*; fenarimol; synthesis of 20-hydroxy ecdysone; cytochrome P-450 inhibitor; microsomal ecdysone 20-monooxygenase.

20-hydroxy ecdysone, the active metabolite of the insect steroid hormone, ecdysone, has multiple functions. It influences not only the regulation of molts, but also other physiological processes during insect development [2]. The precursors of this hormone are usually dietary sterols which are converted into ecdysone via cholesterol [3]. In immature stages the main site of transformation of cholesterol to ecdysone is in the prothoracic glands (in Dipteran species, the ring gland), although other organs can also carry out this conversion which includes several hydroxylation steps. Then, in peripheral tissues, such as the fat body, Malphigian tubules, gut, etc., the secreted ecdysone is converted (hydroxylated) by the cytochrome P-450-dependent ecdysone 20-monooxygenase enzyme system into 20-hydroxy ecdysone.

Injection of triarimol [4], a pyrimidine-containing fungicide (inhibitor of ergosterol biosynthesis [5]), into larvae of *N. bullata* at the wandering stage, caused a delay in the pupariation process. This effect was reversed by 20-hydroxy ecdysone. Later dietary experiments with triarimol [6] and fenarimol [7] showed molting disturbances of *N. bullata*; 0.2% fenarimol added to the diet inhibited the molt to the next stage, causing permanent 1st instar larvae (for about 4–6 days)[8], while at 0.1%, it induced precocious pupariation [10, 11]. Compounds containing nitrogen heterocycles (pyridine, pyrimidine, imidazole or triazole rings) are well known as inhibitors of cytochrome P-450-dependent monooxygenases [12] via reversible ligand formation with heme. Effects caused by fenarimol in vivo suggested that this compound probably acts on the biosynthesis of 20-hydroxy ecdysone.

Materials and methods

Effects of fenarimol (fig. 1) on the biosynthesis of ecdysone were analyzed with undisrupted tissue (i.e. intact cells), while the effects on 20-hydroxylation were measured with a crude enzyme preparation. Larvae of

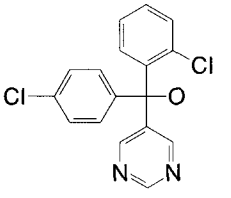

Figure 1. The chemical structure of fenarimol.