LITERATURE CITED

1. L. A. Zakharova and S. V. Sorokin, Modern Methods of Immunotherapy [in Russian], Tashkent (1984), p. 267.
2. Yu. I. Zimin, Progress in Science and Technology. Series: Immunology [in Russian], Vol. 11, Moscow (1983), p. 41.
3. A. A. Zozulya, E. Pacakova, and N. V. Kost, Vest. Akad. Med. Nauk SSSR, No. 1, 28 (1982).
4. A. A. Zozulya, E. Pacakova, N. V. Kost, et al., Mediators of the Immune Response in Experimental and Clinical Medicine [in Russian], Moscow (1983), p. 62.
5. A. A. Zozulya, S. F. Pshennikin, M. R. Shurin, et al., Acta Endocrinol. (Copenhagen), 110, 284 (1985).
6. N. V. Kost, E. Pacakova, and A. A. Zozulya, Biol. Psychiat., 18, 763 (1983).
7. A. A. Mikhailova and L. A. Zakharova, Immunologiya, No. 4, 5 (1985).
8. R. V. Petrov, M. E. Vartanyan, A. A. Zozulya, et al., Byull. Éksp. Biol. Med., No. 5, 46 (1983).
9. J. E. Blalock and E. M. Smith, Fed. Proc., 44, No. 1, 108 (1985).
10. E. G. Fischer and N. E. Falke, Psychother. Psychosom., 42, 195 (1984).
11. S. C. Gilman, J. M. Schwartz, R. J. Milner, et al., Proc. Natl. Acad. Sci. USA, 79, 4226 (1982).
12. E. Hazum, K. J. Chang, and P. Cuatrecasas, Science, 205, 1033 (1979).
13. S. Lolait, A. Lim, B. Foh, et al., J. Clin. Invest., 73, 277 (1984).
14. H. W. McClain, J. B. Lamster, J. M. Bozzone, et al., Life Sci., 31, 1619 (1982).
15. J. Rossier, E. French, R. Guillemin, et al., Neural Peptides and Neuronal Communication, New York (1980), p. 363.

CHANGES IN EFFECTS OF SODIUM ARACHIDONATE IN VITRO AND IN VIVO PRODUCED BY NONSTEROID ANTIINFLAMMATORY AGENTS

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In the modern view an essential role in the mechanism of action of nonsteroid antiinflammatory agents (NSAIA) is played by their inhibitory effect on prostaglandin (PG) biosynthesis [3, 5, 6], in which arachidonic acid is an endogenous precursor [2, 4].

In experiments in vitro and in vivo the writers have compared the effects of various NSAIA [acetylsalicylic acid (aspirin), ibuprofen, dichlofenac sodium, butadione, indomethacin] on the effects of sodium arachidonate (SA), a water-soluble salt of arachidonic acid.

EXPERIMENTAL METHOD

The effect of NSAIA on the spasmogenic effect of SA was studied on isolated segments of the ileum from guinea pigs of both sexes weighing 250-400 g. The tone of the smooth-muscle organs was recorded under isotonic conditions by mechano-electronic transducers (Hugo Sachs Elektronik, West Germany). The NSAIA (10^{-9}-10^{-5} g/ml), dissolved in Ringer's solution, were added to the jar containing the organ 3 min before SA ($5 \cdot 10^{-8}$ g/ml).

The effect of NSAIA on the spasmogenic action of other "mediators" of inflammation [histamine ($5 \cdot 10^{-8}$ g/ml), serotonin ($2 \cdot 10^{-6}$ g/ml), bradykinin (10^{-8} g/ml), PGE_2 ($5 \cdot 10^{-4}$ g/ml)] was studied in experiments on isolated segments of guinea pig ileum, and the effect of NSAIA on the inflammatory action of these "mediators" was studied on a model of acute inflammation

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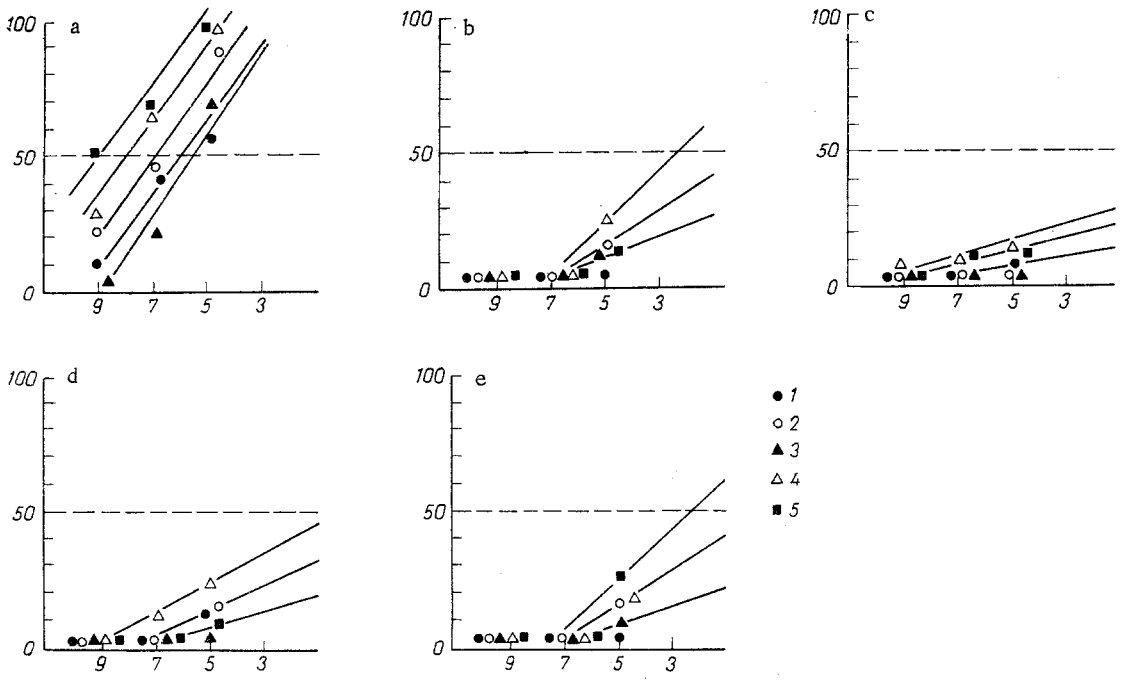

Fig. 1. Action of NSAIA on spasmogenic effect of SA (a), histamine (b), serotonin (c), bradykinin (d), and PGE_2 (e). Abscissa, negative logarithm of concentration of NSAIA; ordinate, inhibition of spasmogenic reaction (in % of control). 1) Aspirin; 2) ibuprofen; 3) butadione; 4) indomethacin; 5) dichlofenac sodium.

of the limb in rats, receiving a subplantar injection of solutions of histamine, bradykinin, and PGE_2 in a concentration of 0.1% and of serotonin in a concentration of 0.01%.

The effect of NSAIA on edema of the limb, due to subplantar injection of 0.1 ml of a 0.1% solution of SA also was studied in vivo. The amount of edema was measured with a plethysmometer (Ugo Basile, Italy). The amount of edema caused by SA in the control animals and in rats receiving NSAIA perorally in equitoxic doses, namely 10% of LD_{50} [1], was compared.

The action of compounds structurally similar to ibuprofen, but, according to our data, not possessing anti-inflammatory activity, also was studied for comparison in experiments in vitro and in vivo on a model of carrageenin edema of the rat limb [6].

EXPERIMENTAL RESULTS

The NSAIA investigated in vitro in concentrations of 10^{-9} to 10^{-6} g/ml caused inhibition to a varied degree of contractions of the isolated guinea pig intestine induced by SA (Fig. 1a). Determination of the inhibitory concentration (IC_{50}) by a graphic method led to the NSAIA being arranged in the following order of their "antiarachidonic" action: diclofenac sodium $\geq$ indomethacin > ibuprofen $\geq$ butadione > aspirin. IC_{50} for these NSAIA was $4 \cdot 10^{-9}$, $1.5 \cdot 10^{-8}$, 10^{-7}, $8 \cdot 10^{-7}$, and $2.5 \cdot 10^{-6}$ g/ml, respectively. The NSAIA studied in concentrations of 10^{-9} to 10^{-7} g/liter caused no change in the spasmogenic effects of histamine, serotonin, bradykinin, and PGE_2 (Fig. 1b-e). With an increase in their concentration to 10^{-5} g/ml, indomethacin, diclofenac sodium, and ibuprofen began to have a weak spasmolytic action, inhibiting contractions induced by these agonists by only 20-25%. Aspirin and butadione as a rule inhibited the spasmogenic effect by not more than 15%.

In experiments in vivo on a model of acute inflammation of the rat limb induced by SA, all the NSAIA studied had a definite anti-inflammatory action, reducing the severity of the inflammatory reaction by 32-82% (Table 1). Meanwhile these NSAIA caused no significant change in acute inflammation induced in rats by histamine, serotonin, bradykinin, or PGE_2. The only preparations with an anti-inflammatory action were diclofenac sodium, which inhibited the inflammatory effect of histamine by 25%, and ibuprofen, which slightly reduced the intensity of the reaction to bradykinin.

TABLE 1. Effect of NSAIA on Acute Inflammation of the Limb Induced in Rats by SA, Histamine, Serotonin, Bradykinin, and PGE_2

Preparation	Dose, mg/kg	SA		Histamine		Serotonin		Bradykinin		PGE_2	
		edema									
		μl	%	μl	%	μl	%	μl	%	μl	%
Control	—	400	100	280	100	1010	100	380	100	360	100
Aspirin	160	270**	68	290	104	870*	86	360	95	310*	86
Ibuprofen	75	220**	55	260	93	1000	99	300**	79	370	103
Butadione	43	260**	65	270	96	1020	101	380	100	410	114
Indomethacin	5	85**	21	240*	86	980	97	320*	84	460*	128
Diclofenac sodium	37	70**	18	210**	75	1100	109	310*	82	400	111

Legend. *$P > 0.05$, **$P \leq 0.05$.

By the intensity of the antiinflammatory effect on a model of acute inflammation in rats induced by SA, the NSAIA could be arranged in the following order: diclofenac sodium $\geq$ indomethacin > ibuprofen $\geq$ butadione $\geq$ aspirin, which is in agreement with the order of their "antiarachidonic" activity in vitro, and also with data on activity of these preparations which the writers obtained on a standard model of acute inflammation induced in rats by carrageenin [1].

The ibuprofen derivatives that were tested, which had no antiinflammatory action but which, according to our data, possess analgesic properties, did not change the action of SA in the above-mentioned doses and concentrations in experiments both in vitro and in vivo.

The investigation shows that NSAIA possess marked "antiarachidonic" activity both in vitro and in vivo. According to the results of the writers' previous investigations [1], in experiments with carrageenin edema of the limb in rats these NSAIA were arranged in the same order of activity as when their "antiarachidonic" action was studied. Compounds closely resembling ibuprofen in their chemical structure, but without any antiinflammatory activity, have no "antiarachidonic" action.

The absence of any significant effect of these NSAIA studied on the spasmogenic action of histamine, serotonin, and bradykinin is evidence of the specificity of their antagonism to the effects of arachidonic acid. The absence of any influence on the effects of PGE_2 is evidence that the action of NSAIA is evidently connected not with any direct influence on the effects of PG, but with inhibition of their formation from arachidonic acid.

It can be concluded from these results that the spasmogenic action of SA in vitro and its proinflammatory effect in vivo (in experiments with edema of the limb in rats) can provide a method of pharmacological screening of chemical compounds for antiinflammatory activity.

LITERATURE CITED

1. G. Ya. Shvarts and R. D. Syubaev, Farmakol. Toksikol., No. 1, 46 (1982).
2. S. Bergstrom, F. Dressler, and L. Krabisch, Arkiv Kemi, 20, 63 (1962).
3. R. Flower, Trends Pharmacol. Sci., 2, 186 (1981).
4. B. Samuelsson, Prostaglandins, 11, 431 (1976).
5. J. R. Vane, Nature, 232, 321 (1971).
6. C. A. Winter, E. Risley, and G. Nuss, Proc. Soc. Exp. Biol. (New York), 111, 544 (1962).