Biochemical Pharmacology, Vol.20, pp. 1355–1361. Pergamon Press, 1971. Printed in Great Britain

EFFECTS OF NON-NARCOTIC ANALGESICS AND NONSTEROID ANTI-INFLAMMATORY AGENTS UPON INORGANIC PHOSPHATES, INTRACELLULAR POTASSIUM AND IMPULSE CONDUCTION IN MAMMALIAN NERVE FIBERS*

M. SCHORDERET† and R. W. STRAUB

Department of Pharmacology, University of Geneva, 20 Rue de l'Ecole de Medecine, CH 1211 Geneva 4, Switzerland

(*Received* 18 *March* 1970; *accepted* 13 *July* 1970)

Abstract—The effects of non-narcotic analgesics and of nonsteroid anti-inflammatory agents on the action potential and the content of intracellular potassium (K_i) and inorganic phosphate (P_i) were studied in the isolated cervical vagus nerve of the rabbit. Acetanilid, phenacetin and antipyrine in concentrations up to 10 mM showed no effect after incubations of 90 min at 37°. On the other hand, the anti-inflammatory agents, salicylic acid, phenylbutazone, oxyphenylbutazone, mefenamic, flufenamic and niflumic acid, in concentrations corresponding to the therapeutic level in human plasma during anti-rheumatic therapy, slowed nervous conduction and finally abolished excitability; this effect was accompanied by a loss of K_i and an increase in P_i. Acetylsalicylic acid was ineffective at concentrations up to 5 mM, and abolished the action potential to 10 mM. Benzydamine, an anti-inflammatory agent without antirheumatic properties, abolished the action potential without elevation in P_i; barbituric acid, furosemide and the complexing agents, cysteamine, D-penicillamine and rubeanic acid, were ineffective. It is concluded that the abolition of excitability observed for the anti-inflammatory agents is due to the K_i loss brought about by interference with oxydative phosphorylation. This effect does not appear to be due to their complexing action on metal ions.

LIM[1,2] HAS shown that non-narcotic analgesics act on the peripheral nervous system and it has been suggested by several authors[3–6] that nonmyelinated nerve fibers (C-fibers) intervene in the generation and propagation of pain. We have, therefore, examined the effects of various analgesic and anti-inflammatory agents on the action potential and on the intracellular content of potassium (K_i) and of inorganic phosphate (P_i) of mammalian nonmyelinated nerve fibers. The nerve used, the cervical vagus of the rabbit, contains, in addition to some myelinated fibers, about 80,000 nonmyelinated fibers with an average diameter of 0·75 μ.[7] It has the advantage that it is relatively easy to remove 10 mg of fresh tissue, so that it is possible to divide the nerve and test the effect of different doses on the same nerve.

EXPERIMENTAL

Cervical vagus nerve of rabbits weighing 2·5–3 kg were removed, desheathed, and weighed immediately after desheathing. The preparations were then placed on plati-

* Send reprint requests to Geneva, Switzerland (address above).

† Present address: Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, Conn. 06510, U.S.A.

num electrodes, stimulated with shocks supermaximal for C-fibers, and shown to conduct impulses by monitoring the compound action potential.

Afterward, the nerves were incubated for 90 min at 37° in Locke solution or in a modified Locke solution containing a drug. Pairs of nerves from the same animal were used; one was incubated in normal Locke solution and served as control. After incubation, the conducting properties of the nerves were re-examined. One-half of the nerve was then placed on perchloric acid (0·5 N), and the inorganic phosphate and protein were determined as previously described.[8] The other half, after being washed for 7 min in K-free Locke, was used for measurements of the K content (see Wespi[9]).

The following substances were used: acetanilid, acetylsalicylic acid, barbital (sodium salt), phenacetin, salicyclic acid (Ph Helv. V), aminopyrine, antipyrine, niflumic acid (U.P.S.A.), cysteamine HCl, D-penicillamine (Fluka), oxyphenylbutazone (sodium salt), phenylbutazone (sodium salt, J. R. Geigy A.G.), gentisic acid (sodium salt), rubeanic acid (Merck), flufenamic acid (sodium salt), mefenamic acid(sodium salt, Parke, Davis & Company), benzydamine (Sauter S. A.), furosemide (Hoechst).

Results are expressed as Δ micromoles P_i per gram of nerve wet weight or in Δ nanomoles P_i per milligram of protein, and in Δ millimoles K per liter of intracellular water,[7] the Δ signifying the differences in the content of P_i or in K_i compared with the values in the control nerves. The standard error of the mean (S.E.) is given whenever appropriate.

RESULTS

In the concentrations used in Table 1, the non-narcotic analgesics, acetylsalicyclic acid, acetanilid, phenacetin and antipyrine, had little or no effect on the P_i content of the isolated nerve preparations, even with concentrations of 5–10 mM, which correspond to the toxic plasma concentrations in man. The action potential, though sometimes reduced, was not abolished, as Fig. 1 illustrates for antipyrine.

On the other hand, salicylic acid in the concentrations obtaining during salicylate therapy in man (2–3 mM[10]) abolished the action potential of both the myelinated (B) and nonmyelinated (C) fibers. With this concentration, there was also an increase in P_i (Fig. 2) and a decrease in K_i. Still more pronounced effects were produced with

TABLE 1. ACTION POTENTIAL AND INORGANIC PHOSPHATE CONTENT AFTER INCUBATION FOR 90 min IN VARIOUS ANALGESICS*

Analgesics	concn. (mM)	pH	Δ μmoles P_i/g wet†	Δ nmoles P_i/mg protein	No. of expts.
Acetylsalicylic acid	5	6·8	+0·08	+0·65	8
Sodium gentisate	10	6·8	+0·19	+1·55	4
Acetanilid	10	6·8	−0·21	−1·71	6
Phenacetin	10	6·8	+0·13	+1·06	4
Antipyrine	10	6·8	−0·16	−1·3	6
Aminopyrine	5	7·4	−0·24	−1·91	6

* Some compound action potential was always present by the end of the incubation period.

† P_i content of 34 control nerves = 1·65 ± 0·17 μmoles P_i/g wet = 18·6 ± 1·4 nmoles P_i/mg protein.

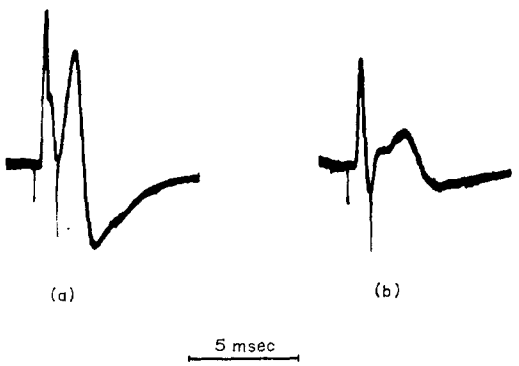

FIG. 1. Compound action potential of desheathed rabbit vagus nerve before (a) and after (b) incubation in 5 mM antipyrine for 90 min at 37°.

higher concentrations (5 and 10 mM). This contrasts with the findings with acetylsalicylic acid, which was ineffective at concentrations up to 5 mM (Table 1); only at a concentration of 10 mM (Fig. 2) was a small, but significant, increase in the content of P_i found, which was accompanied by abolition of the action potential. This effect of 10 mM acetylsalicylic acid may well have been due to the salicylic acid formed by the hydrolysis of the compound during incubation.[11,12]

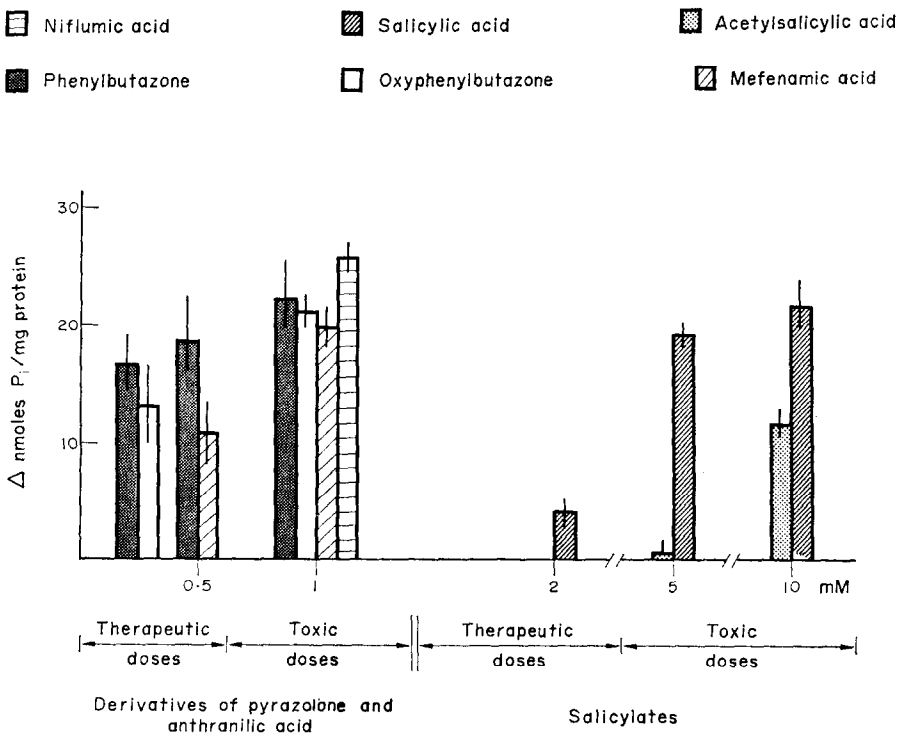

FIG. 2. Effects of anti-inflammatory agents on content in inorganic phosphate in desheathed rabbit vagus nerve. Ordinate indicates increase in phosphate measured after 90 min of incubation at 37°; abscissa shows the concentrations of agents used.

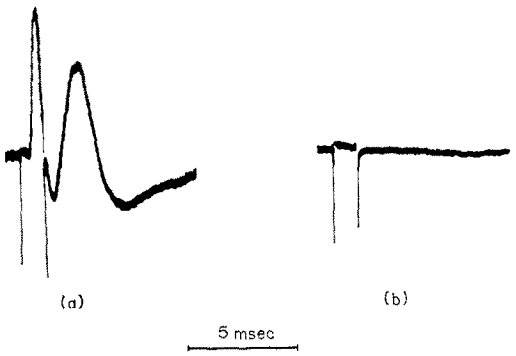

FIG. 3. Compound action potential of desheathed rabbit vagus nerve (a) and abolition of excitability in B and C fibers (b) after incubation in 5 mM phenylbutazone for 90 min at 37°.

The pyrazolone derivatives (phenylbutazone and oxyphenylbutazone) (Fig. 3) and the anthranilic acid derivatives (mefenamic acid, flufenamic acid, niflumic acid) also blocked conduction. This block was accompanied by an increase in the content of P_i and a decrease in the content of K_i (Figs. 2 and 4).

The effect of different concentrations of phenylbutazone in increasing P_i is illustrated in Fig. 2. Phenylbutazone was added to the Locke solution, at concentrations of 0·25, 0·5 and 1 mM; the first value is similar to the therapeutic plasma concentration in man, while the third corresponds to a toxic concentration.

Figures 2 and 4 suggest a certain correlation between the increase of the content in P_i and the decrease in K_i for increasing doses of phenylbutazone and oxyphenylbutazone.

Table 2 shows the effects of barbituric acid, furosemide (a diuretic derived from anthranilic acid) and benzydamine (an anti-inflammatory agent without antirheumatic properties).

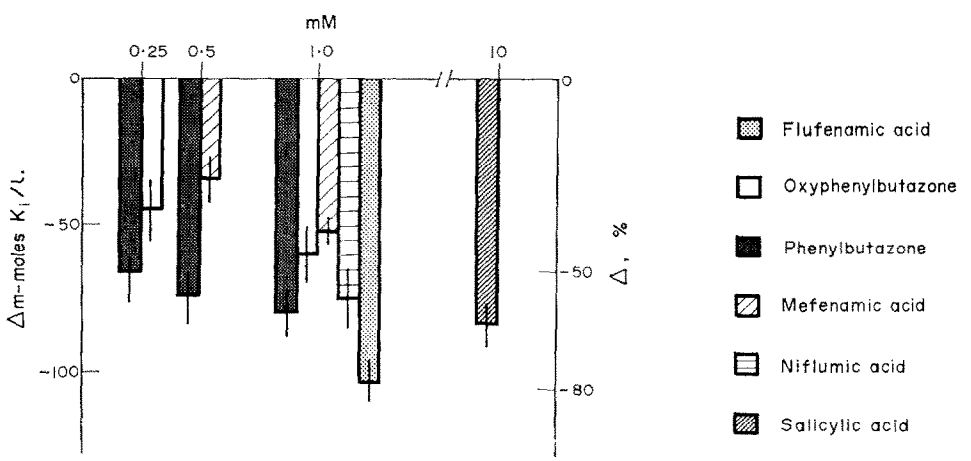

FIG. 4. Effects of anti-inflammatory agents on the potassium content of desheathed rabbit vagus nerve. Ordinate indicates decrease in potassium content after 90 min of incubation, calculated as loss of intracellular potassium or in per cent of initial potassium content; abscissa shows the concentrations of agents used.

TABLE 2. ACTION POTENTIAL AND INORGANIC PHOSPHATE CONTENT AFTER INCUBATION FOR 90 min IN DIFFERENT DRUG SOLUTIONS*

Drug	concn. (mM)	pH	Δ μmoles P_i/g wet	Δ nmoles P_i/mg protein	Δ m-moles K_i/l.	No. of expts.
Barbital	5	6·6	−0·50	−4·1	n.m.†	3
Furosemide	0·1	6·8	−0·54	−4·3	n.m.	2
Benzydamine	0·05	7·2	+0·26	+2·12	−8	4
Benzydamine	0·1	7·2	+0·05	+0·40	−15	4
Benzydamine	0·25	7·2	+0·23	+1·96	+14	4
Cysteamine	1	6·8	+0·23	+1·87	n.m.	2
D-Penicillamine	2	7·1	+0·55	+4·5	n.m.	4
Rubeanic acid	0·1	6·8	+0·68	+5·55	n.m.	2
Rubeanic acid	1	6·8	+0·51	+4·16	n.m.	2

* Except after exposure to benzydamine, some electrical activity could be elicited at the end of the incubation period.

† The abbreviation (n.m.) means not measured.

Benzydamine (Table 2), like the other anti-inflammatory agents tested, abolished the action potential but not necessarily by the same mechanism, since the contents of P_i and K_i were not significantly altered. It should be noted that benzydamine is advocated for the treatment of so-called "primary" inflammation[13] and it would therefore differ from the salicylates, the derivatives of pyrazolone and anthranilic acid, anti-inflammatories and antirheumatics. Table 2 shows that cysteamine and D-penicillamine also produced no effect on P_i.

DISCUSSION

The present experiments on isolated nerve preparations show that phenylbutazone and oxyphenylbutazone produce three probably interdependent effects: an abolition of the action potential, a decrease of intracellular potassium, and an increase in inorganic phosphate. The analgesics acetylsalicylic acid, acetanilid and phenacetin, on the other hand, were completely inactive in nerve trunks; therefore, if they act as suggested by Lim,[1,2] on the peripheral nervous system, their site of action must be confined to the nerve endings.

An interesting difference was found between salicylic acid and acetylsalicylic acid: in concentrations corresponding to therapeutic values, pronounced effects were observed for salicylic acid whereas acetylsalicylic acid was found to be ineffective. The difference in activity between the two compounds could perhaps explain the greater anti-inflammatory action of salicylic acid. It is interesting that 5-hydroxysalicylic acid or gentisic acid, a metabolite of salicylic acid which is sometimes advocated for the treatment of rheumatism,[14, 15] was inactive.

The effects found for salicylic acid were also observed with the pyrazolone and anthranilic acid derivatives; they were also found for the arylacetic acids, indomethacin and fenclozic acid (unpublished observations). All studies with these anti-inflammatory agents on different tissues and subcellular particles[16–21] have agreed in revealing a disturbance of oxidative phosphorylation with concentrations similar to those used therapeutically. Whitehouse[22] has indeed suggested that the uncoupling

properties of these agents are responsible for their antiphlogistic activity, leading to, amongst other phenomena, a modification of the membrane permeability of the inflamed tissues and an inhibition of synthesis of mucopolysaccharides. Although this hypothesis has been criticized by numerous authors (see for example Refs 23 and 24) an uncoupling of the oxidative phosphorylation would provide a plausible explanation for the increase in the P_i content on our isolated nerve; the concomitant decrease of the ATP content would then disturb the K and Na concentration gradients, and so produce inexcitability. We concur with Whitehouse's argument[22] concerning the anomalous behavior of aminopyrine; this substance being a relatively strong base, differs from all the other anti-inflammatory agents, which are acidic in nature, a property which seems to be involved in the ability to uncouple oxidative phosphorylation. Aminopyrine *in vivo* is transformed into acid metabolites (rubazonic acid and *N*-methylrubazonic acid) interfering in turn with the metabolism of the high energy phosphorylated compounds.

The case of benzydamine is interesting in that it does not belong to the category of classic anti-inflammatory agents, its usage being limited to post-traumatic inflammation;[13] like aminopyrine, it is basic, which may account for its inability to uncouple oxidative phosphorylation. Although benzydamine blocks nerve conduction, it probably does so by a different mechanism. This raises the question of whether or not the modification of the conductive properties of the nerve fibers has any relation to the anti-inflammatory activity.

It has been suggested that a number of anti-inflammatory agents possess a certain affinity for heavy metals,[22] due to the presence of ionizable hydrogen; and it has been supposed that this ability to form metallic complexes (in nonaqueous media) could underly the molecular mechanism of the anti-inflammatory activity.[25] Thus, salicylic acid has the property of forming relatively stable complexes with aluminum, iron, copper and uranium.[26] Moreover, it has been proved that thiosalicylic acid and cysteamine inhibit the biosynthesis of mucopolysaccharides,[25] a reaction common to most anti-inflammatory agents. Furthermore, D-penicillamine is sometimes used as an antirheumatic agent.[27–30] However, as Table 2 shows, cysteamine, D-penicillamine and rubeanic acid, substances known to complex metal ions, have no effect on isolated nerve preparations. It is thus rather difficult to affirm definitely that the anti-inflammatory action, *in vivo*, is due to the inhibition of nervous conduction. Nevertheless, this property of antirheumatic drugs on an isolated nerve could serve as a test for anti-inflammatory activity.

Acknowledgements—The authors acknowledge the competent technical assistance of Miss D. Mevissen and the financial support of the Fonds National Suisse de la Recherche Scientifique (Grant No. 4550).

REFERENCES

1. R. K. S. LIM, in *Pharmacology of Pain* (Ed. R. K. S. LIM), Vol. 9, p. 169. Pergamon Press, Oxford (1966).
2. R. K. S. LIM, F. GUZMAN, D. W. RODGERS, K. GOTO, C. BRAUN, G. D. DICKERSON and R. J. ENGLE, *Archs int. Pharmacodyn. Thér.* **152,** 25 (1964).
3. H. LABORIT, *Neurophysiologie, Aspects Metaboliques et Pharmacologiques*, p. 137. Masson, Paris (1969).
4. R. MELZACK and P. D. WALL, *Science, N.Y.* **150,** 971 (1965).
5. A. G. M. WEDDEL, in *The Assessment of Pain in Man and Animals* (Eds. C. A. KEELE and R. SMITH), pp. 47–59. Livingston, Edinburgh (1962).

6. C. A. WINTER, *Analgetics, Chemistry and Pharmacology* (Ed. G. DESTEVENS), p. 22. Academic Press, New York (1965).
7. R. D. KEYNES and J. M. RITCHIE, *J. Physiol., Lond.* **179,** 333 (1965).
8. M. SCHORDERET, *Pharm. Acta Helv.* **44,** 624 (1969).
9. H. H. WESPI, *Pflügers Arch. ges. Physiol.* **306,** 262 (1969).
10. M. J. H. SMITH, and P. K. SMITH, *The Salicylates: A Critical Bibliographic Review*, p. 52. Wiley, New York (1966).
11. M. SCHORDERET, *Abstr. Fourth Int. Cong. Pharmacology*, p. 355. Basel (1969).
12. L. THOMPKINS and K. H. LEE, *J. pharm. Sci.* **58,** 102 (1969).
13. B. SILVESTRINI and S. TURA, *Inflammation, Proc. Int. Symp.* (Eds. B. SILVESTRINI and S. TURA) p. 26. Excerpta Medica Foundation, Amsterdam (1968).
14. K. MEYER and C. RAGAN, *Science, N.Y.* **108,** 281 (1948).
15. L. E. SCHAEFER, I. A. RASHKOFF and R. S. MEGIBOW, *Circulation* **2,** 265 (1950).
16. M. J. H. SMITH and S. W. JEFFREY, *Biochem. J.* **64,** 589 (1956).
17. T. M. BRODY, *J. Pharmac. exp. Ther.* **117,** 39 (1956).
18. S. S. ADAMS and R. COBB, *Nature, Lond.* **181,** 773 (1958).
19. M. W. WHITEHOUSE and J. M. HASLAM, *Nature, Lond.* **196,** 1323 (1962).
20. A. B. FALCONE, R. L. MAO and E. SHRAGO, *Biochim. biophys. Acta* **69,** 143 (1963).
21. D. T. P. DAVIES, R. S. TONKS and A. HUGHES, *Experientia* **25,** 366 (1969).
22. M. WHITEHOUSE, *Prog. Drug Res.* **8,** 321 (1965).
23. H. J. BRÖHR and D. A. KALBHEN, *Archs int. Pharmacodyn. Thér.* **176,** 380 (1968).
24. D. A. KALBHEN, K. EHLERS and R. DOMENJOZ, *Med. Pharmac. exp.* **16,** 552 (1967).
25. M. W. WHITEHOUSE, *Nature, Lond.* **194,** 984 (1962).
26. K. B. YATSIMIRSHII and V. P. VASILOW, *Instability Constants of Complex Compounds*, p. 163, Library of Congress Catalog, New York (1960).
27. I. A. JAFFE, *Arthritis Rheum.* **8,** 1064 (1965).
28. J. ZUCKNER, R. H. RAMSEY, R. W. DORNER and G. E. GANTHER, *Arthritis Rheum.* **10,** 322 (1967).
29. A. LORBER, L. CUTLER and C. C. CHANG, *Arthritis Rheum.* **11,** 65 (1968).
30. B. KLAMER, E. T. KIMURA and M. MAKSTENIEKS, *Pharmacology* **1,** 283 (1968).