EFFECTS OF INDOMETHACIN AND THE OTHER ANTI-INFLAMMATORY AGENTS ON ACTIVATION OF DORSAL HORN CELL IN THE SPINAL CORD INDUCED BY INTRA-ARTERIAL INJECTION OF BRADYKININ

Masamichi SATOH, Takayuki DOI, Kazuo KAWASAKI, Akinori AKAIKE and Hiroshi TAKAGI

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606, Japan

Accepted January 28, 1976

Abstract—Bradykinin (1-2 μg) injected into the femoral artery of the rabbit induced a marked increase in the firing rates of the dorsal horn cells (lamina V cells). This response was markedly inhibited by intravenous administration of indomethacin (1.0 mg/kg), acetylsalicylic acid (20 mg/kg), aminopyrine (50 mg/kg) and oxyphenbutazone (20 mg/kg) in both intact and spinal preparations. Moreover, the intra-arterial injection of these agents depressed the response in lower doses. With these same low doses, no significant suppression occurred when the intravenous route was used. These results indicate that indomethacin and other anti-inflammatory agents tested suppress the bradykinin-induced response of the dorsal horn cell at the periphery, probably at the paravascular sensory nerve.

Previously Satoh et al. (1) have reported that bradykinin injected into the femoral artery of the rabbit induced a marked increase (55% of neurons tested), decrease (20%) or no change (25%) in unit activity of lumbar dorsal horn neurons which were activated by strong mechanical stimuli. Similar results were obtained in the cat by Besson et al. (2). Satoh et al. (1) and Takagi et al. (3) further observed that small doses of morphine (0.3-2.0 mg/kg, i.v.) inhibited activation of dorsal horn lamina V cells by intra-arterial injection of bradykinin in intact, but not in spinal rabbits. Lamina V cells of the dorsal horn have been considered to be involved in transmission of pain (2-5, 16-23).

The present experiments were performed to investigate the effects of indomethacin and other anti-inflammatory drugs on the bradykinin-induced activation of lamina V cells of the dorsal horn in intact or spinal rabbits.

MATERIALS AND METHODS

Fifty-one rabbits, each weighing 2.5-3.0 kg, were anesthetized with ether, immobilized with gallamine triethiodide (Flaxedil), artificially ventilated and placed in a stereotaxic apparatus. The vertebral column was immobilized with metal clamps. Following laminectomy, the dura mater was opened and the cord exposed from L5 to S1, then covered with warm paraffin oil (37-38°C). In some experiments the spinal cord section was performed at L2 level. Recordings were started three hours after termination of the ether anesthesia. Lidocaine was repeatedly applied to wound edges throughout the entire experiments. Ex-
tracellular unit activity was recorded from the left lamina V cells of L6–L7 segments using a tungsten microelectrode, the tip of which was 1–3 \( \mu \)m in diameter. The cells were selected according to the electrophysiological properties described by Wall (4) and Kitahata et al. (5). The potentials were amplified and displayed on an oscilloscope and photographed.

A polyethylene cannula of 0.9 mm in diameter was introduced in a retrograde manner into the left deep femoral artery and tied into place in such a way that the tip was just distal to the bifurcation. Bradykinin (1 or 2 \( \mu \)g in 0.1 ml saline) was rapidly (1 sec or less) injected into the femoral artery through the cannula at intervals of 10 min and the cannula was flushed with saline (0.1–0.2 ml) 1–2 min after each bradykinin injection. In some experiments, drugs were injected through the cannula in order to determine peripheral actions.

When recording the spontaneous activity and the response to arterial bradykinin injection, we used a small computer (Nihon Kohden, ATAC 501–10S) to measure the number of unit activities occurring during second periods. The number of unit discharge of each neuron was counted for 60 sec before and after each bradykinin injection. The number of unit discharges after bradykinin minus the number before bradykinin is considered as bradykinin-induced. The 95 % confidence limits were calculated from five values of the bradykinin-induced discharges before administration of the drug. If the values following two successive bradykinin injections after drug administration were either below or above the calculated 95 % confidence limits, the effect of the drug was regarded as being inhibitory or facilitatory, respectively, and expressed as a percent change from control. To examine effects of drugs, only those neurons in which unitary activity increased following bradykinin injection were given consideration. No tachyphylaxis was observed on the bradykinin-induced response for more than 210 min when this agent injected at intervals of 10 min.

After termination of experiments in which a typical response to bradykinin injection was observed, the location of the recording electrode in the spinal cord was histologically confirmed. Tips of the electrodes were located in the cells which corresponded to the lamina V of cat's dorsal horn (6).

The materials used were: synthetic bradykinin (dissolved in saline), indomethacin sodium or arginate (1% in saline), aminopyrine (5% in saline), acetylsalicylic acid (1.7% in 2% potassium citrate solution), and oxyphenbutazone (3% in weak alkaline solution, pH 8).

RESULTS

Effect of indomethacin

Intravenous injection of 1.0 mg/kg of indomethacin inhibited the bradykinin-induced increases in number of unit discharges of the dorsal horn lamina V cells in the majority of experiments (Table 1), while the same dose of indomethacin had no effect on the spontaneous activities of the dorsal horn cells. Differences in the effects of the drug were not observed between intact and spinal preparations. The effect of 1.0 mg/kg (i.v.) of indomethacin lasted for 60–90 min in either type of preparation. Fig. 1 shows a representative result of experiments using spinal rabbits. Enhancement of the bradykinin-induced unit discharges by
indomethacin was not observed in the present experiments. When 0.05 mg/kg of indomethacin were administered into the femoral artery of intact rabbits through the cannula used for bradykinin injection, the number of bradykinin-induced unit discharges decreased to 5-50% of control in 6 cases out of 8 experiments, whereas intravenous administration of the same dose of the drug resulted in a decrease in 3 cases out of 7 experiments. Similar results were obtained with a dose of 0.1 mg/kg of the drug. These results are summarized in Table 1.

**Effects of other anti-inflammatory agents**

Intravenous administration of acetylsalicylic acid (20 mg/kg) decreased the bradykinin-induced response to 0-40% of control in 3 out of 4 intact and to 35-70% of control in 4 out of 7 spinal rabbits (Table 2), however, there was no effect on the spontaneous activity of

---

**Table 1. Effects of various doses of indomethacin on the bradykinin-induced increases in unit discharges of lamina V cells of the spinal dorsal horn in intact and spinal rabbits**

| Preparations | Doses (mg/kg) | Route of Adm. | Inhibition | Facilitation | No effect |
|--------------|---------------|---------------|------------|--------------|-----------|
| Intact       | 0.05          | i.v.          | 3          | 0            | 4         |
| Intact       | 0.05          | i.a.          | 6          | 0            | 2         |
| Intact       | 0.1           | i.v.          | 2          | 0            | 2         |
| Intact       | 0.1           | i.a.          | 4          | 0            | 1         |
| Intact       | 1.0           | i.v.          | 4          | 0            | 1         |
| Spinal       | 1.0           | i.v.          | 4          | 0            | 1         |

**Fig. 1.** Effect of intravenous administration of indomethacin (1 mg/kg) on the bradykinin-induced increase in unit discharges of lamina V cell of the spinal dorsal horn in a spinal rabbit. Upper row: immediately before bradykinin injection, spontaneous activity. Lower row: about 20 sec after the intra-arterial injection of bradykinin (1 μg; 0.1 ml). Calibration: 500 μV. Time scale: 0.1 sec.
The inhibitory effect of acetylsalicylic acid (20 mg/kg) on the bradykinin-induced response lasted for 60–90 min in intact rabbits and for 40–60 min in spinal rabbits. The inhibitory effect of acetylsalicylic acid (20 mg/kg) on the bradykinin-induced response lasted for 60–90 min in intact rabbits and for 40–60 min in spinal rabbits. Fig. 2 shows a representative result of experiments using spinal rabbits.

When 5 mg/kg of acetylsalicylic acid were injected into the femoral artery of intact rabbits, bradykinin-induced response was inhibited to 5–50% of control in 4 out of 5 experiments, whereas an intravenous injection of the same dose resulted in no apparent change. These results are summarized in Table 2.

### Table 2. Effects of anti-inflammatory drugs on the bradykinin-induced increases in unit discharges of lamina V cells of the spinal dorsal horn in intact and spinal rabbits

| Drugs            | Doses (mg/kg) | Preparations | Inhibition | Facilitation | No effect |
|------------------|---------------|--------------|------------|--------------|-----------|
| Acetylsalicylic acid | 20 (i.v.)     | Intact       | 3          | 0            | 1         |
|                  | 20 (i.v.)     | Spinal       | 4          | 0            | 3         |
|                  | 5 (i.v.)      | Intact       | 0          | 0            | 2         |
|                  | 5 (i.a.)      | Intact       | 3          | 0            | 1         |
| Aminopyrine      | 50 (i.v.)     | Intact       | 3          | 0            | 1         |
|                  | 50 (i.v.)     | Spinal       | 3          | 0            | 1         |
|                  | 10 (i.v.)     | Intact       | 0          | 0            | 2         |
|                  | 10 (i.a.)     | Intact       | 3          | 0            | 0         |
| Oxyphenbutazone  | 20 (i.v.)     | Intact       | 4          | 0            | 1         |
|                  | 20 (i.v.)     | Spinal       | 2          | 0            | 0         |

Fig. 2. Effect of intravenous administration of acetylsalicylic acid (20 mg/kg) on the bradykinin-induced increase in unit discharges of lamina V cell of the spinal dorsal horn in a spinal rabbit. Upper row: immediately before the intra-arterial injection of bradykinin, spontaneous activity. Lower row: about 20 sec after bradykinin (1 µg/0.1 ml) injection. Calibration: 500 µV. Time scale: 0.1 sec. BK: Bradykinin.
Intravenous administration of aminopyrine (50 mg/kg) decreased the number of bradykinin-induced responses of the dorsal horn cells to 10-25% of control in 3 cases out of 4 experiments with intact rabbits, and to 20-60% of control in 3 out of 4 spinal rabbits (Table 2), while the same dose of aminopyrine had no effect on the spontaneous activities of the dorsal horn lamina V cells. These depressant effects lasted for 60-90 min in both preparations. A similar depression of the bradykinin response of the dorsal horn cells was observed after intra-arterial injection of 10 mg/kg of this drug (injection rate; 2-3 min/ml), but not after an intravenous administration of 10 mg/kg. Enhancement of the bradykinin-induced unit discharges by acetylsalicylic acid and aminopyrine was not detected in the present experiments (Table 1).

Intravenous administration of oxyphenbutazone (20 mg/kg) decreased the bradykinin-induced response of dorsal horn cells to 20-50% of control in intact as well as spinal rabbits (Table 2). This effect had a slow onset (20-25 min) and a long duration (120-180 min). There was no effect on the spontaneous activities of dorsal horn cells.

DISCUSSION

Anti-inflammatory agents such as indomethacin and aminopyrine have generally been considered to have analgesic action only in conditions where pain accompanies an inflammatory response (7). Clinical studies, however, have shown that indomethacin is most effective as an analgesic in patients with acute pain such as postoperative or post-traumatic pain in which inflammatory reactions are absent (8).

Bradykinin has been postulated to be a possible candidate responsible for the production of pain in humans (9-11) and in animals (12-15). Moreover bradykinin-induced unit discharges of the dorsal horn cells (lamina V) were proved to be inhibited after injections of small doses of morphine (1, 3). Many electrophysiological studies have demonstrated that the dorsal horn cells are involved in the transmission of nociceptive message to the upper centers (16-23) and inhibited by some anesthetics and analgesics (1, 23-27).

Our findings herein give a tentative pharmacological basis for the analgesic actions of indomethacin and the other anti-inflammatory drugs tested and indicate that the site of analgesic action of indomethacin and related drugs is mainly in the periphery, since small doses of these drugs inhibited the bradykinin-induced response after intra-arterial injections. In contrast, morphine has been shown to depress the lamina V cells through activation of the descending inhibitory system from the supra-spinal center (1, 3).

The above findings are in accord with those of Lim et al. (14, 28) who showed that non-narcotic analgesics (acetylsalicylic acid, aminopyrine and phenylbutazone) block the bradykinin-induced "pseudo-affective pain response" at the periphery, probably the paravascular sensory nerves and also lend further support to the thesis of Winter and Flataker (29) that indomethacin has true analgesic action aside from its anti-inflammatory action in rats. Nevertheless, our results do not rule out the possibility that the analgesic actions of indomethacin and related drugs may to some extent be mediated through a central effect.

Acknowledgements: This work was supported by grants from the Naito Foundation.
Indomethacin sodium or arginate was kindly provided by Sumitomo Chemical Co., Ltd., Osaka, Japan.

REFERENCES

1) SATOH, M., NAKAMURA, N. AND TAKAGI, H.: Europ. J. Pharmacol. 16, 245 (1971)
2) BESSON, J.M., CONSEILLER, C., HAMAN, K.-F. AND MAILLARD, M.-C.: J. Physiol. 221, 189 (1972)
3) TAKAGI, H., SATOH, M., DOI, T., KAWASAKI, K. AND AKAIKE, A.: Archs int. Pharmacodyn. Thér. (in press)
4) WALL, P.D.: J. Physiol. 188, 403 (1967)
5) KITAHATA, L.M., TAUB, A. AND SATO, I.: J. Pharmacol. exp. Ther. 176, 101 (1971)
6) REXED, B.: J. comp. Neurol. 96, 415 (1952)
7) WOODBURY, D.M.: The Pharmacological Basis of Therapeutics, Edited by GOODMAN, L.S. and GILMAN, A., p. 314, Macmillan Company, London (1970)
8) BEAVER, W.T.: Am. J. med. Sci. 250, 577 (1965)
9) ARMSTRONG, D., DRY, R.M.L., KEE, C.A. AND MARKHAM, J.W.: J. Physiol. 120, 326 (1953)
10) ARMSTRONG, D., JEPSON, J.B., KEE, C.A. AND SILWART, J.W.: J. Physiol. 135, 350 (1957)
11) BURCH, G.E. AND DE PASQUALE N.P.: Circulation Res. 10, 105 (1962)
12) GUZMAN, F., BRAUN, C. AND LIM, R.K.S.: Archs int. Pharmacodyn. Thér. 136, 353 (1962)
13) GUZMAN, F., BRAUN, C., LIM, R.K.S., POTTER, G.D. AND RODGERS, D.W.: Archs int. Pharmacodyn. Thér. 149, 571 (1964)
14) LIM, R.K.S., GUZMAN, F., RODGERS, D.W., GOTO, K., BRAUN, C., DICKFISON, G.D. AND ENGEL, R.J.: Archs int. Pharmacodyn. Thér. 152, 25 (1964)
15) HASHIMOTO, K., KUMAKURA, S. AND Taira, N.: Japan. J. Physiol. 14, 299 (1964)
16) MENDEL, L.M. AND WALL, P.D.: Nature 206, 97 (1965)
17) WALL, P.D.: Anesthesiology 28, 46 (1967)
18) POMERANZ, B., WALL, P.D. AND WEBER, W.V.: J. Physiol. 199, 511 (1968)
19) HILLMAN, P. AND WALL, P.D.: Exp. Brain Res. 9, 284 (1969)
20) SELZER, M. AND SPENCER, W.A.: Brain Res. 14, 331 (1969)
21) SELZER, M. AND SPENCER, W.A.: Brain Res. 14, 349 (1969)
22) WAGMAN, I.H. AND PRICE, D.D.: J. Neurophysiol. 32, 803 (1969)
23) DE JONG, R.H. AND WAGMAN, I.H.: Exp. Neurol. 20, 352 (1968)
24) DE JONG, R.H., ROBLES, R. AND MORIKAWA, K.: Anesthesiology 31, 205 (1969)
25) DE JONG, R.H., ROBLES, R. AND HEAVNER, J.E.: Anesthesiology 32, 440 (1970)
26) CONSEILLER, C., BENOSTI, J.M., HAMANN, K.F., MAILLARD, M.C. AND BESSON, J.M.: Europ. J. Pharmacol. 18, 346 (1972)
27) BESSON, J.M., WYON-MAILLARD, M.C., BENOSTI, J.M., CONSEILLER, C. AND HAMANN, K.F.: J. Pharmacol. exp. Ther. 187, 239 (1973)
28) LIM, R.K.S.: Pharmacology of Pain, Edited by LIM, R.K.S., ARMSTRONG, D. AND PARDO, E.G., p. 169, Pergamon Press, Oxford (1968)
29) WINTER, C.A. AND FLATAKER, L.: J. Pharmacol. exp. Ther. 150, 165 (1965)