INHIBITORY MECHANISMS OF DEXAMETHASONE ON CONTRACTIONS INDUCED BY DRUGS AND BY TRANSMURAL STIMULATIONS IN ISOLATED GUINEA PIG ILEUM

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Abstract—Inhibitory effects of dexamethasone on contractile response of isolated ileum of guinea pig were isotonically investigated. Responses to picric acid and transmural stimulation at 50 Hz were employed for stimulations of cholinergic nerve and responses to potassium chloride and transmural stimulation at 1 Hz were taken as stimulation of smooth muscle, while response to acetylcholine was considered as stimulation of receptor sites. Dexamethasone significantly attenuated the contractile responses to cholinergic nerve stimulation in concentrations relatively lower than were required to depress the responses to muscle stimulations. The specific action of dexamethasone on receptor sites was not, however, responsible for this result as the dose-response curve of acetylcholine-induced contractions was shifted by dexamethasone to downward but not to the right side. Since the effect of dexamethasone was not modified in the reserpinized tissues and was not affected by treatment with tolazoline or propranolol, participation of adrenergic mechanisms in the effect of dexamethasone was ruled out. In addition, the effects of dexamethasone on both responses to nerve and muscle stimulations were reduced in a high calcium solution and potentiated in a low calcium solution. These results suggest that dexamethasone affects cholinergic nerve endings rather than acting on smooth muscle cells, and that interference with the movement of calcium ions is responsible for the inhibitory effect of dexamethasone.

It has been reported that anti-inflammatory drugs of either the steroid type (1) or the non-steroid type (2) inhibit the contraction of smooth muscle produced by various stimulating drugs. Mechanisms of inhibitory action of non-steroid compounds on vascular smooth muscle have been studied in detail (3). Mechanism of the inhibitory effect of steroid compounds in intestinal smooth muscle, however, remains obscure. In the present study, inhibitory mechanisms of dexamethasone on the contractile responses of the isolated guinea pig ileum to transmural stimulations and various drugs were investigated.

MATERIALS AND METHODS

Guinea pigs of both sexes, weighing between 300 and 350 g, were stunned and bled and ileal strips were prepared. Each segment approximately 1.5 cm in length was suspended in a 30 ml organ bath containing Locke solution maintained at 28±1°C and continuously bubbled with a mixture of 95% O₂ and 5% CO₂. The nutrient solution used had the following composition (mM): NaCl 154, KCl 5.6, CaCl₂ 2.2, glucose 5.6 and NaHCO₃ 4.8 (pH
7.4). Tissues were equilibrated for 90 minutes in nutrient solution before any measurement was taken and the solutions were replaced every 30 minutes.

An electronic square-pulse generator (Nihon Kohden, MSE-3R) was used to stimulate the tissue transmurally via parallel bipolar platinum electrodes, 1.0 cm apart. The entire whole ileal strip was placed between two platinum wires. Electrical stimulations lasting 5 seconds and of submaximal voltage (50 V) were applied every 30 minutes. Two types of stimulation, pulse width of 1 msec and 100 msec, were employed.

Drug solutions were added dropwise into the bath media and washed out with nutrient solutions. Contractile responses were isotonically recorded on smoked drums by levers with a ten-fold amplification. All responses were expressed as a percentage of the control responses. Statistical difference was calculated by means of paired Student’s t-test, unless otherwise stated.

Drugs used were as follows: acetylcholine chloride (Tokyo Kasei, Japan), atropine sulfate (Merck, Germany), dexamethasone sodium phosphate (Merck-Banyu, Japan), hexamethonium bromide (Tokyo Kasei, Japan), tolazoline hydrochloride (Yamanouchi, Japan), nicotine (Tokyo Kasei, Japan), physostigmine salicylate (Merck, Germany), picric acid (Wako Chemicals, Japan), potassium chloride (Wako Chemicals, Japan), propranolol hydrochloride (Sumitomo Chemicals, Japan), reserpine (Daichi, Japan) and tetrodotoxin crystalline 3X (Sankyo, Japan).

RESULTS

Contractile responses to nicotine, picric acid, potassium chloride, acetylcholine and transmural stimulations

Addition of nicotine (5 × 10^-7 to 6 × 10^-5 g/ml), picric acid (2 × 10^-5 to 10^-4 g/ml), potassium chloride (KCl) (5 × 10^-1 to 3 × 10^-8 g/ml) and acetylcholine (ACh) (10^-5 to 10^-6 g/ml) produced dose-dependent contractions. 10^-6 g/ml nicotine, 5 × 10^-5 g/ml picric acid, 10^-3 g/ml KCl and 5 × 10^-9 g/ml ACh with an approximate ED50 were used. Transmural stimulation of ileal strips of guinea pig produced a contraction that was increased with the increased pulse frequency from 0.1 Hz to 60 Hz at the pulse width of 1 msec. Submaximal response to stimulations at 10 Hz, 1 msec, and the approximate ED50 response to stimulations at 10 Hz, 1 msec, were employed.

Contractile responses to nicotine, picric acid and transmural stimulations at 10 Hz and 50 Hz were markedly reduced or abolished by 10^-7 g/ml atropine and 10^-7 g/ml tetrodotoxin, while such were significantly increased by 10^-4 g/ml physostigmine. Contractile response to ACh was completely abolished by 10^-7 g/ml atropine, was increased by 10^-8 g/ml physostigmine but was not affected by 10^-7 g/ml tetrodotoxin. However, the responses to 10^-4 g/ml KCl and transmural stimulation at 1 Hz, 100 msec, were not modified by any drugs used in this experiment. These results are summarized in Table 1.

Treatment with hexamethonium in a concentration (2 × 10^-5 g/ml) sufficient to abolish the contractile response to nicotine did not affect the response to picric acid and slightly decreased responses to transmural stimulations at 10 Hz and 50 Hz. The contractile re-
### Table 1. Effects of atropine, tetrodotoxin and physostigmine on the contractile responses to nicotine, picric acid, potassium chloride, acetylcholine and transmural stimulations in isolated guinea pig ileum

| Treatment            | Nicotine 10⁻⁶ g/ml | Picric acid 5 × 10⁻³ g/ml | KCl 10⁻³ g/ml | Acetylcholine 5 × 10⁻⁸ g/ml | 10 Hz, 1 msec Transmural stimulations | 50 Hz, 1 msec | 1 Hz, 100 msec |
|----------------------|--------------------|---------------------------|---------------|-----------------------------|--------------------------------------|--------------|---------------|
| Non-treatment        | 100                | 100                       | 100           | 100                         | 100                                  | 100          | 100           |
| Atropine 10⁻⁷ g/ml   | 1.2 ± 0.3 (8)*     | 0 (8)*                    | 100.1 ± 0.3 (8)* | 0 (8)*                      | 0 (6)*                               | 0 (8)*       | 99.4 ± 1.6 (8) |
| Tetrodotoxin 10⁻⁷ g/ml | 2.3 ± 0.4 (8)*     | 0 (8)*                    | 100.1 ± 0.1 (8)* | 100.6 ± 0.4 (8)             | 0 (6)*                               | 0 (6)*       | 100.6 ± 1.2 (9) |
| Physostigmine 10⁻⁵ g/ml | 113.2 ± 1.6 (10)* | 124.6 ± 2.1 (6)*          | 100.9 ± 0.6 (8) | 116.8 ± 2.1 (9)*           | 118.7 ± 2.1 (6)*                     | 121.8 ± 0.9 (8)* | 100.7 ± 2.2 (9) |

Number of preparations used is expressed in parentheses. Values (means ± S.E.) are expressed as percentage of the control response. *: Significant difference from respective controls, p < 0.01.

### Table 2. Effect of dexamethasone on contractile responses to picric acid, potassium chloride and transmural stimulations in isolated guinea pig ileum

| Treatment            | Picric acid 5 × 10⁻³ g/ml | KCl 10⁻³ g/ml | Transmural stimulations |
|----------------------|---------------------------|---------------|--------------------------|
|                      | 100                       | 100           | Transmural stimulations  |
| Non-treatment        | 100                       | 100           | 100                      |
| Dexamethasone (g/ml) |                           |               |                          |
| 10⁻⁴                 | 86.1 ± 2.1 (8)*           | 98.7 ± 2.1 (8) | 74.6 ± 2.4 (8)*         |
| 2 × 10⁻⁴             | 72.1 ± 1.8 (8)*           | 99.7 ± 0.6 (8) | 68.1 ± 4.1 (8)*         |
| 3 × 10⁻⁴             | 62.7 ± 2.1 (8)*           | 98.5 ± 1.3 (8) | 60.7 ± 1.6 (9)*         |
| 4 × 10⁻⁴             | 53.2 ± 2.5 (9)*           | 86.4 ± 6.1 (6)* | 54.4 ± 2.7 (9)*        |
| 8 × 10⁻⁴             | 32.6 ± 1.0 (9)*           | 62.8 ± 2.2 (8)* | 34.6 ± 1.8 (8)*        |
| 10⁻¹                 | 11.3 ± 2.6 (8)*           | 54.9 ± 1.6 (8)* | 55.4 ± 0.8 (8)*       |

Number of preparations used is expressed in parentheses. Values (means ± S.E.) are expressed as percentage of the control response. Transmural stimulations at 10 Hz and 50 Hz were used to stimulate the cholinergic nerves and 1 Hz, 100 msec was for stimulation of the muscle cells. *: significant difference from respective controls, p < 0.01.
sponse to KCl was also unaffected by the treatment with tolazoline or propranolol in a concentration (10^{-7} g/ml or 5 \times 10^{-7} g/ml, respectively) sufficient to abolish the submaximal responses of norepinephrine or isoproterenol.

From these results, responses to picric acid and transmural stimulations at 10 Hz, 1 msec and 50 Hz, 1 msec, were used for the cholinergic nerve stimulations whereas the responses to KCl and transmural stimulation at 1 Hz, 100 msec, were taken as the direct stimulation of smooth muscle in the following experiments.

**Effect of dexamethasone on the contractile responses to picric acid, potassium chloride, acetylcholine and transmural stimulations**

Treatment with dexamethasone significantly attenuated the contractile responses to picric acid and transmural stimulations at 10 Hz and 50 Hz was dose-dependent in concentrations ranging from 10^{-3} to 10^{-4} g/ml. Inhibitory effect of dexamethasone on the transmural stimulation-induced contractions was greater in preparations stimulated at lower pulse frequency, i.e. 10 Hz, 1 msec, than that stimulated at 50 Hz (Table 2). Response to electrical stimulation at 10 Hz, 1 msec, was about 48\% of the maximal response of transmural stimulation at 60 Hz, 1 msec. The addition of dexamethasone in a dose over 4 \times 10^{-5} g/ml also suppressed the contractile responses to KCl and transmural stimulation at 1 Hz, 100 msec.

Otherwise, contractile response to ACh was also reduced by dexamethasone in a dose higher than 4 \times 10^{-5} g/ml and the dose-response curve of ACh-induced contractions recorded by the cumulative dose method was not parallely shifted to the right but only downward. (Fig. 1).

![Fig. 1. Effect of dexamethasone on the dose-response curve of ACh-induced contractions.](image-url)

*Fig. 1.* Effect of dexamethasone on the dose-response curve of ACh-induced contractions. ○ ○: control, ▲ ▲: treatment with 4 \times 10^{-5} g/ml dexamethasone, ■ ---■: treatment with 10^{-5} g/ml dexamethasone. Vertical bars represent standard errors of means obtained from 8 experiments.
As shown in Table 3, the inhibitory effect of dexamethasone on the contractile responses to picric acid and KCl was not modified by treatment with $10^{-7}$ g/ml tolazoline or $5 \times 10^{-7}$ g/ml propranolol. In addition, the inhibitory effect of dexamethasone also appeared in the ileal strips of guinea pig treated with reserpine 0.3 mg/kg 24 hours before sacrifice. There was no significant difference in inhibition between the reserpinized ileum and the untreated one.

**Influence of external calcium ions on the inhibitory effect of dexamethasone**

As shown in Fig. 2-A, $2 \times 10^{-5}$ g/ml dexamethasone suppressed the contractile response to transmural stimulation at 50 Hz, 1 msec, to 68.1% of the control value. The inhibitory effect of dexamethasone in the Ca-modified bath medium was compared with that in normal bath medium by means of the unpaired Student’s t-test. The inhibitory effect of dexamethasone was prevented when external Ca was increased to 2.7 mM, while such was markedly enhanced when external Ca was lowered to 1.0 mM. Similarly, the inhibitory effect of $4 \times 10^{-5}$ g/ml dexamethasone on the contractile response to transmural stimulation at 1 Hz, 100 msec, was markedly enhanced by lowering of external Ca concentrations and prevented by raising of external Ca concentrations. (Fig. 2-B).

The dose-response curve for contraction of guinea pig ileum by picric acid was carried out by the cumulative dose method and that by KCl was obtained by the single dose method. Dexamethasone $2 \times 10^{-5}$ g/ml shifted the dose-response curve of picric acid to downward. (Fig. 3). This downward shift was prevented by raising the external Ca concentrations and enhanced by lowering these concentrations.

In addition, $4 \times 10^{-5}$ g/ml dexamethasone shifted the dose-response curve of KCl-induced
Fig. 2. Influence of external Ca concentrations on the inhibitory effect of dexamethasone on contractile responses to transmural stimulations at 50 Hz (A, the left side) and at 1 Hz (B, the right side). Response to transmural stimulation in normal media containing 2.2 mM Ca was taken as 100% for each other. The blank columns represent the response of untreated tissues and the lined columns represent the response of dexamethasone treated tissues. The rate of inhibition by dexamethasone compared from each response of untreated tissue in Ca-modified bath medium was significantly different from that in normal bath medium (*: unpaired Student’s t-test, P<0.02). Vertical bars represent standard errors of means from 10 experiments.

Fig. 3. Influence of external Ca in the inhibitory effect of dexamethasone on the dose-response curve of picric acid-induced contractions. Response to picric acid recorded by cumulative dose method in normal media (containing 2.2 mM Ca) was expressed as △—△ and that in Ca-rich media (containing 2.7 mM Ca) was ■—■, while that in Ca-poor media (containing 1.0 mM Ca) was ○. Effect of 2×10⁻⁵ g/ml dexamethasone in each different bath media was expressed as △, △, △, △ and ○, respectively. The contractile response to picric acid in normal media initiated at 10⁻³ g/ml was taken as 100%. Vertical bars represent standard errors of means from 8 experiments.

Fig. 4. Influence of external Ca on the inhibitory effect of dexamethasone on the dose-response curve of potassium chloride (KCl). Response to KCl recorded by single dose method in normal media (containing 2.2 mM Ca) was expressed as △—△ and that in Ca-rich media (containing 4.7 mM Ca) was ■—■, while that in Ca-poor media (containing 0.5 mM Ca) was ○. Effect of 4×10⁻⁵ g/ml dexamethasone in each different bath media was expressed as △, △, △ and ○, respectively. Contractile response to normal KCl at 3×10⁻³ g/ml was taken as 100%. Vertical bars represent standard errors of means from 8 experiments.
contractions downward. Similarly, the downward shift of the dose-response curve by dexamethasone was also prevented by raising the external Ca concentrations and enhanced by lowering these concentrations. (Fig. 4).

**DISCUSSION**

We found herein that dexamethasone inhibited the contractile responses to drugs and transmural stimulations. Dexamethasone more markedly depressed the contractile responses to picric acid and transmural stimulations at 10 Hz, 1 msec, and 50 Hz, 1 msec, than those to KCl, ACh and transmural stimulation at 1 Hz, 100 msec. Otherwise, the responses to picric acid and transmural stimulations at 10 Hz and 50 Hz were blocked by the treatment with atropine as well as tetrodotoxin and augmented by physostigmine, suggesting that those responses are mediated through the release of ACh from cholinergic nerve endings. Takagi and Takayanagi (4) reported that picric acid liberates ACh from nerve endings to induce contractions on the small intestine of guinea pig. Our observations herein substantiate these findings (4). Responses to KCl and transmural stimulation at 1 Hz, 100 msec, are considered to be produced by direct stimulation of smooth muscle as such responses are not modified by the treatment with tetrodotoxin or atropine. In general, electrical stimulation at low pulse width has been used as a method of eliciting neurotransmitter release in a variety of tissues (5, 6).

The inhibitory effect of dexamethasone was obtained on the contractile responses to picric acid and transmural stimulations at 10 Hz and 50 Hz. In addition, from the findings that larger doses of dexamethasone are required to alter the dose-response curve of ACh-induced contractions than the doses required to block the response to cholinergic nerve stimulations, and that the dose-response curve of ACh-induced contractions was not parallely shifted by dexamethasone to the right but only downward, it is may be considered that selective affinity of dexamethasone to the cholinergic receptor sites was negligible in production of the inhibitory effect. Our findings suggest that dexamethasone may produce an inhibitory effect on the cholinergic nerve endings in relatively lower doses and then also act on the myogenic sites of guinea pig ileum in higher doses.

Bass and Setliff (1) reported inhibitory effects of various steroid compounds on the isotonic responses to nicotine, pilocarpine, histamine etc., in the guinea pig ileum and suggested that steroids act on the surface of the cell or at some point within the cell membrane. However, the inhibitory effect of dexamethasone on the neurogenic sites has not been here- tofore considered.

In vascular smooth muscle, steroids had a potentiating effect on responses to catecholamine (7, 8). The present study revealed that the inhibitory effect of dexamethasone on the responses to drugs of the ileum isolated from reserpinized animals is not significantly different from that on the responses of non-treated tissues, and that effect of dexamethasone was not modified by the treatment with tolazoline or propranolol. Thus, participation of adrenergic mechanisms in the action of dexamethasone can be ruled out.

Moreover, it is generally considered that Ca ions are required for the release of neuro-
transmitters (9) and for the contraction of muscles (10). In the present study, we also found that inhibitory effects of dexamethasone, either on the neurogenic sites or on the myogenic sites, were enhanced in a low Ca medium and were reduced in a high Ca medium (Figs. 2–4). Therefore, it is suggested that inhibition of the movement of Ca ions on the nerve endings or the muscle cell sites is involved in the inhibitory mechanisms of dexamethasone on the contractions induced by various drugs and transmural stimulations in the isolated guinea pig ileum.

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REFERENCES

1) Bass, A.D. and Setliff, J.A.: The in vitro actions of steroids on smooth muscle. J. Pharmacol. exp. Ther. 130, 469–473 (1960)
2) Jaques, R. and Domenjoz, R.: Histaminantagonistische Wirkung bei Pyrazolen und Antihistaminen. Arch. exp. Path. Pharmacol. 212, 124–134 (1950)
3) Northover, B.J.: Mechanism of the inhibitory action of indomethacin on smooth muscle. Brit. J. Pharmacol. 41, 540–551 (1971)
4) Takagi, K. and Takayanagi, I.: Liberation of acetylcholine from the small intestines of guinea pigs and cats after treatment with picric acid. Nature 193, 589–590 (1962)
5) Hollingsworth, M.: Mechanical responses of rat isolated uterine horns to transmural stimulation. Brit. J. Pharmacol. 55, 41–46 (1975)
6) Paton, W.D.M. and Zar, A.M.: The origin of acetylcholine released from guinea-pig intestine and longitudinal muscle strips. J. Physiol. 194, 13–33 (1968)
7) Besse, J.C. and Bass, A.D.: Potentiation by hydrocortisone of responses to catecholamines in vascular smooth muscle. J. Pharmacol. exp. Ther. 154, 224–238 (1966)
8) Yard, A.C. and Kadowitz, P.J.: Studies on the mechanism of hydrocortisone potentiation of vasoconstrictor responses to epinephrine in the anesthetized animal. Europ. J. Pharmacol. 20, 1–9 (1972)
9) Harvly, A.M. and Macintosh, F.C.: Calcium and synaptic transmission in a sympathetic ganglion. J. Physiol. 97, 408–416 (1940)
10) Ebashi, S. and Endo, M.: Calcium ions and muscle contraction. Prog. Biophys. Mol. Biol. 18, 123–183 (1968)