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# ROLE OF CALCIUM IN THE ANALGESIC EFFECT OF NONNARCOTIC ANALGESICS AND CALCIUM CHANNEL BLOCKERS

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Evidence has recently been published that calcium ions are involved directly or indirectly in the control of nociceptive sensitivity [4, 5, 10]. For instance, the antinociceptive action of various inorganic and organic calcium antagonists has been demonstrated [6, 8, 10]. Meanwhile, no attempt has yet been made to analyze data on quantitative relationships between the strength of the analgesic action of different substances and their ability to block calcium transport.

The aim of this investigation was to compare the analgesic action of calcium antagonists (verapamil, fenigidin) and the promising nonnarcotic analgesic PV-107 with their effect on calcium transport.

## EXPERIMENTAL METHOD

The analgesic activity of the substances was studied by the hot plate test [9]. Experiments were carried out on noninbred male albino mice weighing 22 ± 2 g, kept on a standard diet and receiving food and water ad libitum. The test substances, namely verapamil (USSR), fenigidin (East Germany), and PV-107 (a promising nonnarcotic analgesic developed at the Kiev Research Institute of Pharmacology and Toxicology, Ministry of Health of the Ukrainian SSR) were dissolved in isotonic sodium chloride solution with the addition of 5% Tween-20, and injected intramuscularly into animals in a volume of 0.2 ml.

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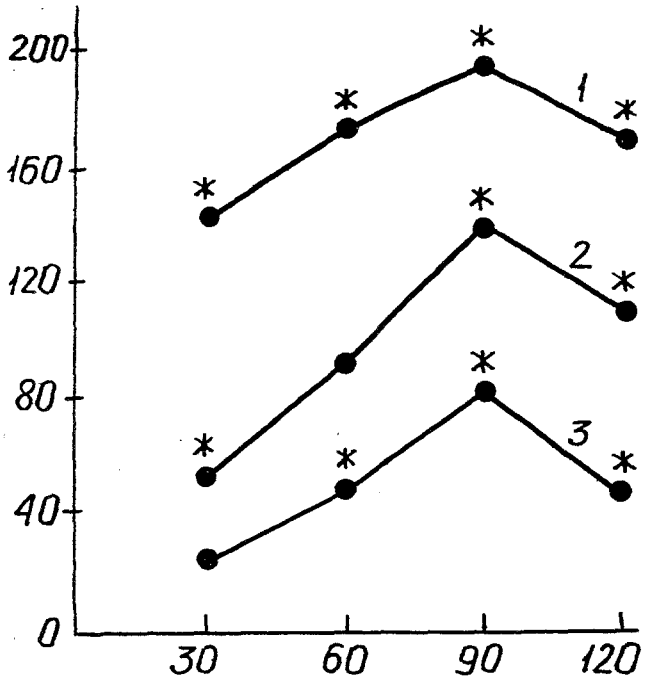


Fig. 1

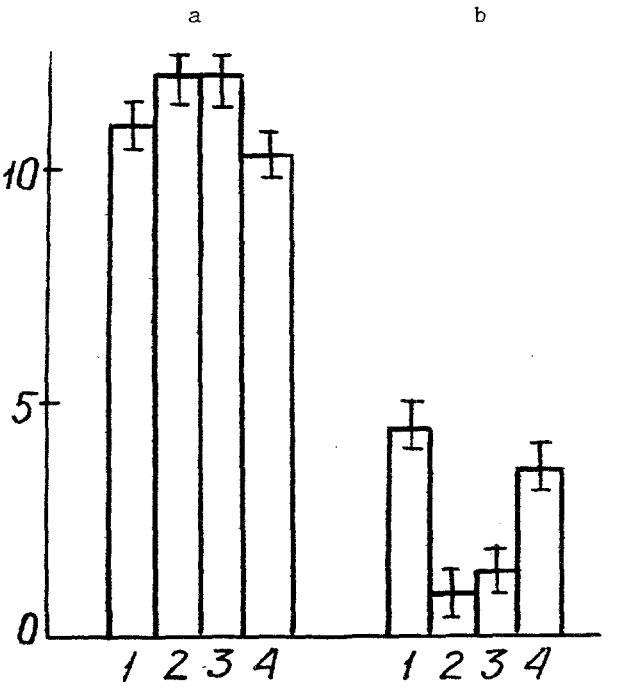


Fig. 2

Fig. 1. Effect of verapamil, fenigidin, and PV-107 on nociceptive sensitivity in mice. Abscissa, time (in min) ordinate, changes in latent period of response (in % of normal): 1) verapamil (0.03 mmole/kg), 2) fenigidin (0.04 mmole/kg), 3) PV-107 (0.03 mmole/kg). *$p < 0.05$.

Fig. 2. Release of $Ca^{2+}$ from vesiculated preparation of rabbit myocardial sarcolemma under conditions of polarization and subsequent depolarization of the membrane. Abscissa: a) polarization, b) depolarization, 1) control, 2) verapamil, 3) fenigidin, 4) PV-107 Ordinate, release of $^{45}Ca^{2+}$ (in nmoles/mg protein) Initial accumulation of $^{45}Ca^{2+}$ in vesicles averaged 24.5 nmoles/mg protein.

TABLE 1. Comparison of Analgesic and Ca-Blocking Activity of Verapamil, Fenigidin, and PV-107

| Substances tested | Analgesic activity, $ED_{50}$, mmole/kg | Ca-blocking activity, percentage inhibition of $Ca^{2+}$ release |
|---|---|---|
| Verapamil | 0,0026 (0,0019–0,0034) | 77 |
| Fenigidin | 0,0101 (0,0071–0,0145) | 64 |
| PV-107 | 0,0129 (0,0077–0,0221) | 22 |

There were two groups of experiments. In the experiments of group 1 the time of onset of the maximal analgesic effect was determined after a single injection of verapamil, fenigidin, and PV-107 in doses of 0.029, 0.043, and 0.028 mmoles/kg respectively. In the experiments of group 2, at the peak of analgesia, $ED_{50}$ was calculated by probit analysis [1].

The effect of verapamil, fenigidin, and PV-107 on passive transport of $^{45}Ca^{2+}$ through voltage-dependent Ca-channels was studied in experiments in vitro on vesiculated preparations of the myocardial sarcolemma. The presence of a membrane potential on the vesiculated sarcolemma in a $K^+$-valinomycin system was demonstrated previously with the aid of the voltage-sensitive probe dis-$C_3$-(5) [2].

Membrane potential-dependent transport of $^{45}Ca^{2+}$ in the vesicles was studied in accordance with the scheme suggested by Dunn [7]. For this purpose, vesicles loaded with labeled $Ca^{2+}$ (5 mM) in low potassium medium (5 mM), were initially introduced into a polarizing medium containing a high $K^+$ concentration (150 mM) and valinomycin, then in depolarizing medium with a low $K^+$ concentration (5 mM) and with valinomycin. Depolarization promotes opening of Ca-channels, which is expressed as additional release of $Ca^{2+}$ compared with release of the cation in a polarizing medium.

The experimental results were subjected to statistical analysis [3].

## EXPERIMENTAL RESULTS

The study of the dynamics of the analgesic action of the preparations showed that the analgesic action of verapamil and PV-107 reached a peak 90 min after intramuscular injection, compared with 120 min in the case of fenigidin (Fig. 1).

The compounds differed significantly in analgesic activity: verapamil > fenigidin > PV-107 (Table 1).

The study of the effect of the compounds on $Ca^{2+}$ release (in mmoles/mg protein) in polarizing medium for 5 min and in depolarizing medium for 20 min showed that all the preparations tested, in a concentration of $10^{-4}$ M, have virtually no effect on release of $^{45}Ca^{2+}$ from the vesicles in polarizing medium (the voltage was −90 mV). Meanwhile, inhibition of $^{45}Ca^{2+}$ release was observed under depolarization conditions, when the membrane voltage fell to −27 mV. However, the inhibitory effect of the compounds differed (Fig. 2). Verapamil and fenigidin blocked $Ca^{2+}$ release by 77 and 64% respectively, whereas PV-107 blocked it by 22% (Table 1).

Comparison of the analgesic and Ca-blocking action of verapamil, fenigidin, and PV-107 revealed clear correlation (coefficient of correlation 0.82) between the analgesic activity of the substances in the "hot plate" test and their Ca-blocking activity under depolarization conditions.

The results can serve as the basis for creation of a new trend in the search for effective nonnarcotic analgesics.

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