Experimental

Ni-PO——Ni-PO was prepared from nickel sulfate and its available oxygen-content was determined by iodometry as reported in the previous paper.[3] Its quantity used in stoichiometric oxidation was calculated on the basis of the available oxygen-content.

Oxidation of Aromatic Aldehydes——Unless otherwise stated, the oxidation of the aromatic aldehydes detailed in Table II were carried out in a following procedure of benzaldehyde.

Oxidation of Benzaldehyde——To a solution of benzaldehyde (5.30 g) and sodium hydroxide (2.5 g) in 125 ml of water was added 19.3 g of Ni-PO (1.2 times the theoretical amount) and stirred for 90 minutes at 30° under nitrogen. The reaction mixture was filtered through a glass filter, Ni-PO was washed with water. The combined filtrate was extracted by ether to remove the unreacted benzaldehyde and acidified with dilute sulfulic acid. The solution deposited 5.75 g (94.3%) of benzoic acid.

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Studies on Isolated Smooth Muscle Cells. II.[1] Potentiation of Calcium Contraction of Isolated Smooth Muscle Cells from *Vas Deferens* of Guinea Pig by Cocaine

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Single smooth muscle cells were isolated from *vas deferens* of guinea pig and effect of cocaine on calcium contraction of the individual cells under partially depolarized condition was examined. Among the isolated cells, a few cells were contracted by 20 mM calcium chloride in medium containing 60 mM potassium chloride. Cocaine increased the ratio of contracted cells. The result suggested that cocaine facilitated calcium contraction of individual cells in the tissue and induced larger contraction of the tissue.

Keywords——cocaine; smooth muscle; smooth muscle cells; isolated cells; guinea pig; *vas deferens*

It is well known that cocaine potentiates various pharmacological responses.[3-7] It was also reported from this laboratory that cocaine potentiated calcium contraction of partially depolarized *vas deferens* of guinea pig.[8] Two mechanisms could be considered for the potentiation: 1) cocaine facilitates propagation of electrical excitation and induces synchronization of contraction of each muscle cell in the tissue which resulted in larger contraction of tissue, or 2) cocaine facilitates contraction of individual cells in the tissue and induces larger contraction. In order to clarify which mechanism worked for the potentiation, effect of cocaine on contraction of individual cells isolated from *vas deferens* of guinea pig was examined.

A male albino guinea pig weighing approximately 300 g was killed with a blow and a pair of *vas deferens* were isolated. The tissue was allowed to stand in calcium-free modified Tyrode solution (2.7 mM KCl, 137 mM NaCl, 1.0 mM $MgCl_2$, 5.6 mM glucose and 6.0 mM $NaHCO_3$) for 90 min at 30°. The medium was gently stirred by aeration and was changed by fresh

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medium every 15 min. The tissue was then suspended in incubation medium (60 mM KCl, 80 mM NaCl, 1.0 mM $MgCl_2$, 5.6 mM glucose and 6.0 mM $NaHCO_3$) for 60 min at 30° with gentle stirring by aeration. The tissue was sliced and the slices were incubated with 0.2% collagenase (Sigma, Type I) for 30 min at 30°. The incubation was terminated by washing the slices and cells were dispersed by pipetting gently through wide bore Pasteur pipette. Dispersed cells were separated from undigested tissue by filtration through nylon mesh and the filtrate was subjected to the experiment. The cell preparation was kept at room temperature (regulated at 20°) and used within 90 min. Details of the single cells were previously reported.[1]

Incubation for calcium contraction of the isolated cells was carried out on silicon-coated slide glass. The incubation was started by mixing 0.05 ml of the cell suspension containing 5—10 cells with 0.1 ml of incubation medium containing 30 mM $CaCl_2$ and 5 mM ATP on the slide glass. As soon as the suspension was mixed with the incubation medium, the slide glass was placed on a stage of phase contrast microscope and cells were continuously observed during the incubation. Incubation was carried out at 20° and the medium was stirred by vibrating the glasses. Degree of contraction was determined photographically. Calcium contraction in the presence or absence of cocaine was examined alternatively in order to avoid effect of aging of cells on the contraction during the experiment. Although ATP was not effective for the calcium contraction, it was added in the incubation medium since it coagulated broken cells and made easy for intact cells to be identified.

When the isolated cells were incubated with 20 mM $CaCl_2$ under partially depolarized condition, almost of the cells contracted little or slightly but a few cells contracted largely. Figure 1 shows time course of contraction of individual cells during the incubation. Cells

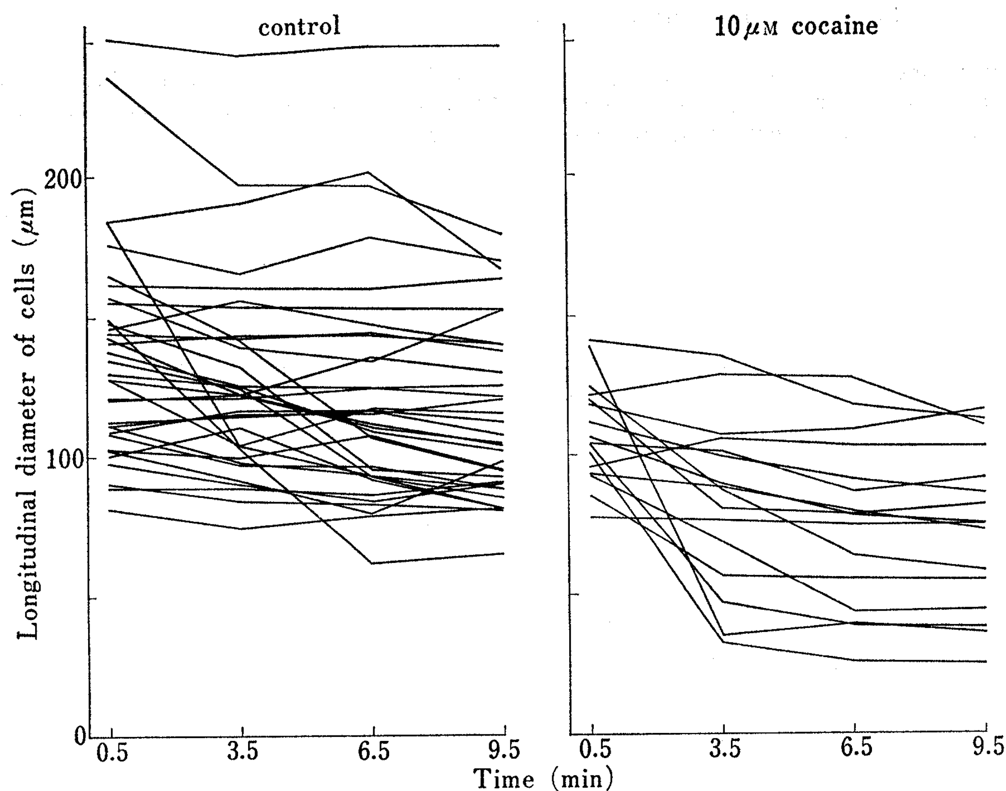

Fig. 1. Effect of cocaine on time course of calcium contraction of isolated cells under partially depolarized condition. Details of the experimental conditions are described in the text. The first determination of the cell size was carried out 0.5 min after the incubation started. Preincubation with cocaine was carried out in test tube for more than 10 min. Time course shown in this figure is a part of result in the series of this experiment.

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which contracted largely completed the contraction within a few minutes. One hundred and five cells were examined and 8 cells contracted largely under the condition. Ratio of the contracted cells to the examined cells was 7.6% as presented in Table I. Presence of 10 μM cocaine hydrochloride, which potentiated calcium contraction of *vas deferens* as a whole tissue under the same condition, resulted in increase in number of largely contracted cells as shown in Fig. 1 and ratio of contracted cells to examined cells was approximately 4 times of that of control experiment as shown in Table I. Presence of 0.3 μM cocaine also increased the ratio but 1.0 nM was not effective.

Table I. Effect of Different Concentration of Cocaine on Calcium Contraction of Isolated Cells

Final concentration (M) of cocaine	Number of cells		% (contracted/examined)
	Examined	Contracted[a]	
None	105	8	7.6
1×10^{-9}	18	1	5.5
3×10^{-7}	16	4	25.0
1×10^{-5}	41	12	29.3

a) Contracted cell represents a cell which becomes 50 μm shorter than the original size during incubation of the first 3 min.
Detailed condition of the experiment is described in Fig. 1.

These results suggested that cocaine facilitated calcium contraction of individual cells and increased ratio of largely contracted cells in the tissue. This might be one of mechanisms of potentiation of calcium contraction by cocaine.

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