Participation of Protein Synthesis in Development of Supersensitivity in Cultured Rat Vas Deferens

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Abstract—Organ culture of rat vas deferens produced supersensitivity to norepinephrine and acetylcholine in contractile response without change in $\alpha_1$-adrenergic and muscarinic acetylcholine receptors. The development of supersensitivity was inhibited by low temperature and protein synthesis inhibitors. However, protein synthesis inhibitor had no significant effect on the receptors. These findings suggested that the supersensitivity may be induced by synthesis of protein(s) which have a stimulatory effect in a process(es) after activation of receptor to contraction.

Supersensitivity caused by denervation is a well-known phenomenon, especially in striated muscles (1, 2). Recently, some information about denervation supersensitivity of the smooth muscle have also been accumulated (3-5), but the mechanism involved in the supersensitivity still remains unclear. We found that rat vas deferens became supersensitive to norepinephrine (NE) and acetylcholine (ACh) by organ culture. To find some clues on the mechanism of the supersensitivity, we examined effects of protein synthesis inhibitors on the development of the supersensitive state induced by organ culture.

Male Sprague-Dawly rats (250-300 g) were decapitated and whole vasa deferentia were isolated aseptically and cultured in Eagle's MEM with 5% calf serum under a passively loaded tension (320 mg/muscle), as reported before (6). Organ culture was carried out at 37°C under 95% O$_2$ and 5% CO$_2$. Cultured muscles were mounted and washed in Locke's solution for 30 min and then contractile responses were measured. The resting tension was adjusted to 500 mg/muscle, and then contractions in response to NE and ACh were recorded isometrically with a force-displacement transducer (SB1T, Nihon Kohden) and recorders. After contractile responses had been recorded, the muscles were weighed and homogenized in 100 vol. of ice-cold medium (50 mM Tris-HCl, pH 7.4, and 10 mM MgCl$_2$) in a Polytron and sonicated at Power 5 for 30 sec in a Kontes sonicator. The suspensions were filtered through nylon mesh (φ 100 μm) to remove connective tissues and used for binding assays. $\alpha_1$-Adrenoceptor ($\alpha_1$-R) was estimated with $^3$H-prazosin (18.0 Ci/mmol) and muscarinic cholinergic receptor (mAChR) was measured with L-$^3$H-quinuclidinyl benzilate (QNB) (33.1 Ci/mmol). The homogenate (4 mg wet weight) was incubated in 1 ml of medium (50 mM Tris-HCl, pH 7.4, and 10 mM MgCl$_2$) with $^3$H-prazosin at 25°C for 30 min and L-$^3$H-QNB at 37°C for 60 min. After the incubation, the homogenate was trapped on a glass filter (Whatman GF/F), and its radioactivity was counted in a liquid scintillation counter. Specific bindings of $^3$H-prazosin and L-$^3$H-QNB were calculated by subtracting non-specific binding in the presence of 10$^{-5}$ M phentolamine and 10$^{-5}$ M atropine from total binding in the absence of these compounds. At the concentrations of 0.1-2 nM $^3$H-prazosin and 0.06-2 nM L-$^3$H-QNB, Scatchard plots of the specific bindings yielded straight lines, respectively. Since the specific binding sites for $^3$H-prazosin at 2.0 nM and L-$^3$H-QNB at 2.0 nM were close to
the maximal number of binding sites, respectively, the specific bindings at this concentration were used for determining the maximal number of $\alpha_1$-R and mACHR. The $K_i$ value for NE and ACh were corrected for the radioligand occupancy shift according to the equation $K_i = K_o \times (1 - L/K_o)$, where $K_o$ and $L$ represent the dissociation constants and the concentrations of $^3$H-prazosin and L-$^3$H-QNB, respectively. Statistical analyses were made by Student’s $t$-test, and a level of $P<0.05$ was regarded as significant.

The contractions to norepinephrine (NE) and acetylcholine (ACh) of rat vas deferens were mediated by $\alpha_1$-R and mACHR on the smooth muscle, respectively, as shown in the other reports (7, 8). As reported previously (6), the supersensitivity of the smooth muscle was induced by organ culture of rat vas deferens. The supersensitivity to NE was constituted with decrease in ED50 value of contraction to NE (the ED50 value after culture for 3 days was 0.48±0.11 $\mu$M, whereas that before culture was 6.8±0.9 $\mu$M) and increase in maximal contraction to NE. The supersensitivity well-resembled the denervation supersensitivity (4, 5). The nature of contraction to NE did not change during culture and was still mediated through $\alpha_1$-R; during development of the supersensitivity of contraction to NE, the density of $\alpha_1$-R in rat vas deferens was measured by binding of the $\alpha_1$-selective antagonist $^3$H-prazosine. The amount of $\alpha_1$-R did not change significantly during organ culture for 3 days, without any change in the $K_o$ value of $^3$H-prazosin (0.32±0.08 nM mean±S.D.). At the same time, the $K_i$ value of NE from the displacement of bound $^3$H-prazosin by NE was not changed (0.59±0.14 $\mu$M). Similarly, a slight increase in maximal contraction to ACh was also observed by organ culture. The increase in mACHR mediated contraction was not accompanied by increase in the amount or change in binding characteristics of mACHR as detected by $^3$H-QNB binding. The $K_o$ value for $^3$H-QNB (38±7 pM mean±S.D.) and the $K_i$ value of $^3$H-QNB for ACh (0.61±0.27 $\mu$M) were not changed during culture. Therefore, the supersensitivity induced by organ culture was not due to quantitative and qualitative changes of the receptors.

Organ culture of rat vas deferens at 4°C did not produce the supersensitivities to NE and ACh, indicating that the development of supersensitivities were temperature-dependent. Furthermore, development of supersensitivity to NE by organ culture of rat vas deferens under 95% air and 5% CO2 was less than that under 95% O2 and 5% CO2. These findings indicated that aerobic metabolic processes are involved in the development of supersensitivity during organ culture. Then, we investigated the effects of protein synthesis inhibitors on this supersensitivity. As shown in Table 1, 50 $\mu$g/ml cycloheximide or 10 $\mu$g/ml puromycin inhibited the development of the supersensitivity in $\alpha_1$-R and mACHR mediated contractions, but these treatments did not affect the numbers of $\alpha_1$-R and mACHR in the tissue. The inhibitors of protein synthesis had no direct effect on the contractions by NE, and ACh and the contraction to high K+ was not significantly affected by the culture with or without the inhibitor of protein synthesis. Thus, the above findings indicated that protein synthesis was involved in the increase in the $\alpha_1$-R and mACHR mediated contractions. The organ culture described above with the inhibitor of protein synthesis was carried for 24 hr, because when the culture period was longer than 1 day, a non-specific toxic effect was observed with inhibitors of protein synthesis.

In receptor-mediated responses, one of the important regulatory mechanisms is on the receptor level. For example, in nicotinic AChR (nACHR) and mACHR systems, the regulation of the response to ACh is very dependent on the change in the number of nACHR and mACHR (1, 2, 9-11). In contrast, in the $\alpha_1$-R system of rat vas deferens, $\alpha_1$-R-mediated contraction of the smooth muscle was dependent on the change(s) in processes after activation of the receptor, rather than a change in the receptor itself. One reason for this phenomenon may be very slow turnover of $\alpha_1$-R in the smooth muscle of rat vas deferens. Actually in our preliminary work, the half-life of $\alpha_1$-R in this muscle was long and estimated to be over 5 days.

The supersensitivity in contraction to NE
and ACh during organ culture was inhibited by low temperature or protein synthesis inhibitors. These findings showed that the supersensitivity was due to temperature-dependent mechanism(s) such as protein synthesis. However, we have already reported that the NE-induced desensitization in the contraction to NE of rat vas deferens might be due to some protein synthesis through activation of \( \alpha \)-adrenoceptors, and its synthesis may induce the decrease in \( \alpha \)-R mediated contraction (6, 12). The data in the present and previous works showed that the sensitivity in contraction to NE through \( \alpha \)1-R could be regulated by complicated mechanisms in which syntheses of various proteins with opposite actions are involved. Further studies are required to elucidate the regulatory mechanisms through these proteins in receptor-mediated contraction in smooth muscle.

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### Table 1. Effects of protein synthesis inhibitors on the supersensitivity during organ culture

| (A) | Maximal contraction (g/g tissue) | | ACh |
|-----|---------------------------------|----|----------------|
| Preculture | 23±2 (18)                      | 11±1 (22) |
| Cultured with | | | |
| No inhibitor | 36±3\( ^a \) (18) | 16±2\( ^a \) (12) |
| Cycloheximide 50 \( \mu \)g/ml | 20±5\( ^b \) (8) | 10±1\( ^b \) (8) |
| Puromycin 10 \( \mu \)g/ml | 18±1\( ^b \) (4) | 11±2\( ^a \) (12) |

| (B) | Binding sites (pmol/g tissue) | \( ^3 \)H-prazosin | \( ^3 \)H-QNB |
|-----|-------------------------------|----------------|-------------|
| Preculture | 10.4±1.4 (11) | 22.0±2.4 (11) |
| Cultured with | | | |
| No inhibitor | 11.3±2.4 (8) | 19.5±3.9 (8) |
| Cycloheximide 50 \( \mu \)g/ml | 11.0±2.1 (8) | 21.5±3.9 (8) |
| Puromycin 10 \( \mu \)g/ml | 11.6±1.7 (4) | 18.8±3.3 (4) |

Rat vasa deferentia were cultured with or without 50 \( \mu \)g/ml cycloheximide or 10 \( \mu \)g/ml puromycin for 1 day. After culture, the contractions to NE and ACh were measured isometrically. (A) Maximal contractions. The amplitude of maximal contractions from dose-response curves are shown as means±S.E. (n). (B) The amounts of \( \alpha \)-R and mAChR. The densities of receptors were measured as the specific binding sites of \( ^3 \)H-prazosin (2.0 nM) and \( ^3 \)H-QNB (2.0 nM), respectively. Data represent means±S.D. (n). These experiments were done by unpaired tests. Significantly different from the preculture level: \( ^a \)P<0.01, Significantly different from value of no inhibitor: \( ^b \)P<0.01, \( ^c \)P<0.05

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