The results obtained herein appear to confirm the observations of other investigators (1-7) that certain morphine protein conjugates can produce antibodies with the specificity, which may differ depending on the used hapten. Also indicated is the possibility of production of the morphine-3-glucuronide specific antibody which would be useful for developing a sensitive urine test for opiate users.

Acknowledgement: The authors wish to thank Sankyo Pharmaceutical Co. Ltd. for providing naloxone and oxymorphone.

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EXTERNAL CALCIUM IONS AND MECHANICAL RESPONSES OF THE AORTA ISOLATED FROM THE CHOLESTEROL- FED RABBIT

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Accepted August 14, 1973

It has been reported that the endoplasmic reticulum in the aorta of the cholesterol-fed rabbit became more extensive than that observed in normal smooth muscle cells (1). Tests have therefore been carried out to determine whether or not the endoplasmic reticulum appearing in the aorta of the cholesterol-fed rabbit plays an important role in contraction of the isolated aorta induced by agonists, while comparing the modes of action of agonists tested on the aorta isolated from the cholesterol-fed rabbit with those tested on the aorta from the normal rabbit.

One group of male albino rabbits was fed a diet containing 2% (w/w) cholesterol (Nihon Crea, CR-2) for 65 to 70 days. The other group was fed a diet without cholesterol. Total amount of serum cholesterol was estimated according to the method originally described by Zak (2) and Henley (3), was 1930.2 ± 90.4 mg/kg (mean $\pm$ S.E.). The fact that the endoplasmic reticulum became more extensive in the aortic smooth muscle was con-

firmed by electron microscopic observations.

After sacrificing a rabbit (3.6 to 4.1 kg in body wt.) by a blow on the neck, a section of the thoracic aorta between the heart and the diaphragm was removed and helically cut strips were prepared according to the method of Furchgott (4). A piece of the aorta was suspended in a 30 ml organ bath filled with the normal Locke Ringer's solution, kept at 32°C and bubbled with air. The normal Locke Ringer's solution used herein contained, in one litre, 9.0 g of NaCl, 0.4 g of KCl, 0.2 g of CaCl₂, 0.2 g of MgCl₂, 0.5 g of NaHCO₃ and 0.5 g of glucose. Responses of the aorta to drugs were isotonically recorded. A concentration action curve of an agonist was cumulatively obtained: first, the curve was obtained in the normal Locke Ringer's solution, then in the Ca-free Locke Ringer's solution after 60 min incubation of the same aorta with the Ca-free Locke Ringer's solution. Ca-free Locke Ringer's solution was made as follows; CaCl₂ was omitted from the normal Locke Ringer's solution and 0.1 mM GEDTA (glycoletherdiamine tetraacetic acid) was added. The experimental results are expressed as a mean of 8 experiments.

TABLE 1. The pD₂ values (mean ± S.E.) and intrinsic activities (mean ± S.E.) of the agonists tested on aortas, isolated from both normal and cholesterol-fed rabbits, suspended in the normal and Ca-free Locke Ringer's solutions.

	Normal rabbit				Cholesterol fed rabbit			
	normal solution		Ca-free solution		normal solution		Ca-free solution	
	i.a.*	pD ₂	i.a.*	pD ₂	i.a.*	pD ₂	i.a.*	pD ₂
Phenylephrine	1.00	6.5 ± 0.11	0.4 ± 0.03	6.0 ± 0.10	1.00	6.8 ± 0.10	0.4 ± 0.07	6.1 ± 0.11
Histamine	1.1 ± 0.08	5.6 ± 0.11	0.1 ± 0.02	5.2 ± 0.10	1.0 ± 0.09	5.9 ± 0.07	0.2 ± 0.01	5.6 ± 0.16
KCl	0.7 ± 0.03	2.6 ± 0.06	0.5 ± 0.03	2.3 ± 0.02	0.8 ± 0.07	2.8 ± 0.06	0.5 ± 0.03	2.2 ± 0.06
Acetylcholine	0.6 ± 0.03	6.0 ± 0.16	0	—	0.4 ± 0.12	5.6 ± 0.16	0	—

* i.a. intrinsic activity—the maximum response to a drug, the maximum response to phenylephrine in a normal solution.

Activity of an agonist was expressed as a pD₂ value. The pD₂ value represented the negative logarithm of the concentration (g/ml) of the agonist that induces a 50% contraction in the isolated aorta. The maximum action of the agonist is expressed as the intrinsic activity according to Ariens (5). Table 1 shows the pD₂-values and intrinsic activities of phenylephrine, histamine, KCl and acetylcholine tested on the aortas isolated from normal and cholesterol-fed rabbits which were suspended in the normal and Ca-free Locke Ringer's solution. No significant differences were observed among all the pD₂-values and intrinsic activities tested on the normal aorta and those tested on the aorta from the cholesterol-fed rabbit. The intrinsic activities of acetylcholine and histamine were greatly reduced by removal of the external Ca-ions, however, phenylephrine and KCl elicited a markedly greater contraction of aorta suspended in Ca-free Locke Ringer's solution than did histamine or acetylcholine. Hudgins and Weiss (6) reported that the responses of the aorta to KCl (25 mM or ca. 1.8×10^{-3} g/ml) were almost abolished by removal of the external Ca ions. The concentration of KCl necessary to produce the maximum con-

traction of the aorta suspended in Ca-free Locke Ringer's solution was 1 to 2×10^{-3} g/ml in our experiments. The response to this high concentration of KCl still remained in Ca-free Locke Ringer's solution. The minor response to the lower concentrations in our experiments (for example, 2×10^{-3} g/ml) was also almost completely abolished by removal of the external Ca-ions. The difference between our findings and that of the aforementioned is presumably due to the concentrations of KCl. It can be concluded from our experimental results that the maximum actions of KCl and phenylephrine are less dependent on the external Ca-ions than are those of histamine and acetylcholine. Since the pD_2 -values and intrinsic activities of the agonists, even KCl and phenylephrine which were less dependent on the external Ca ions, were not significantly modified by administration of cholesterol to the test animal (Table 1), the endoplasmic reticulum observed in the aorta of the cholesterol-fed rabbit is considered not to play an important role such as Ca store in the contraction mechanism of the isolated aorta.

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EFFECT OF L-GLUTAMINE ON INDOMETHACIN-INDUCED GASTRIC LESIONS IN THE RAT

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Accepted October 29, 1973

In a previous study from this laboratory it was indicated that L-glutamine exerts a strong inhibitory effects on aspirin-induced gastric lesions in rats (1). Similar to aspirin, indomethacin is well known to evoke a side effect of gastrointestinal damages in humans (2) and animals (3-5). Whether or not L-glutamine has a beneficial effect on gastric damage in rats as induced by indomethacin was thus investigated herein.

Male Donryu strain rats, weighing 165-175 g prior to experiments, were fasted for 24 hr except for free access to water. Indomethacin (20 mg/kg), suspended in a trace of Tween 80, was given either i.p. or per os to intact or pylorus-ligated rats 10 min after the operation. The pylorus was ligated under ether anesthesia according to the standard method by Shay et al. (6). L-glutamine suspended in 1% carboxymethylcellulose (CMC) solution was given orally over a wide dose range ten min before indomethacin adminis-