L'indométhacine semblerait agir par antagonisme direct. Elle se comporterait ainsi comme d'autres anti-inflammatoires, les fénamates, l'aspirine et la phénylbutazone qui inhibent l'action de la $PGF_{2\alpha}$ sur les bronches humaines in vitro[12].

Comme l'indométhacine n'inhibe que l'hypotension artérielle tardive sans modifier l'hypertension qui la précède, on serait conduit à admettre que la $PGF_{2\alpha}$ se fixe chez le Rat sur deux types de récepteurs vasculaires au moins: les premiers qui sont initialement les plus actifs, commandent la vasoconstriction. Leur action est labile et sensible aux processus de tachyphylaxie. Les seconds sont responsables de la vasodilatation. Ils seraient différents des récepteurs à la PGE_2 puisque l'action hypotensive de cette dernière n'est pas modifiée par l'indométhacine. Toutefois JONES[13] estime qu'un seul type de récepteurs vasculaires serait responsable de la vasodilatation induite par les PG: il serait activé principalement par la PGE_2. Aussi, une autre éventualité reste à discuter: l'hypotension dépendrait de la conversion de la $PGF_{2\alpha}$ en PGE_2 ou en un autre facteur hypotenseur. Certains tissus du Rat possèdent une 9 oxo-réductase qui catalyse la transformation de PGE_2 en $PGF_{2\alpha}$ et vice versa[14,15]. L'antagonisme exercé par l'indométhacine résiderait alors dans le blocage de cette étape métabolique, l'anti-inflammatoire interférant avec plusieurs enzymes qui interviennent dans la synthèse et le métabolisme des PG[16,17] dont la 9 oxo-réductase.

Cette enzyme serait activée progressivement par la $PGF_{2\alpha}$ tout comme par les PG dérivées de l'acide arachidonique ou encore par celles mises en circulation par la bradykinine[18]; celle-ci, de plus, active une 9 oxo-réductase isolée à partir d'artères mésentériques de Bœuf[19]. Cette dernière interprétation: la conversion de la $PGF_{2\alpha}$ en PGE_2 nous paraît s'inscrire le mieux dans la biochimie et la pharmacologie des PG.

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Ouabain-Induced Release of Extraneuronal Catecholamine in the Isolated Guinea-Pig Vas Deferens

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Summary. It was found that ouabain (10^{-5} *M*) was effective in releasing the extraneuronal catecholamine which was taken up by $uptake_2$ process in the guinea-pig vas deferens. This result shows that a Na-K ATPase is essential for the storage of catecholamine in the extraneuronal site.

IVERSEN[1] originally described the existence of two mechanisms of uptake of catecholamine in the isolated perfused rat heart; $uptake_1$ (neuronal uptake) and $uptake_2$ (extraneuronal uptake). $Uptake_2$ appeared in several aspects to differ from $uptake_1$. In contrast to $uptake_1$, $uptake_2$ was usually inoperable at the low perfusion concentration of catecholamine, and the catecholamine taken up by $uptake_2$ process was washed out time-dependently if the perfusion was continued with catecholamine-free medium[1-3]. Recently, it was found that metanephrine[1], clonidine[4] and various steroids, including deoxycorticosterone (DOC)[5] and cholesterol[6], were effective to inhibit selectively the $uptake_2$ in the perfused rat heart. However, the physiological significance of this $uptake_2$ and its release processes are still obscure. We have suggested in the preceding paper[7] that ouabain, a typical inhibitor of Na–K ATPase, might facilitate the release of catecholamine from the neuronal site in the guinea-pig vas deferens. The purpose of the present study has been to determine whether ouabain is effective in facilitating the release of catecholamine in extraneuronal site stored by $uptake_2$ process in the isolated guinea-pig vas deferens.

Materials and methods. Male guinea-pigs weighing 300–500 g were used. A piece of vas deferens was dissected and suspended in a bath containing Tyrode solution (32 °C). The contraction was recorded isometrically on an inkwriting polygraph through a force-displacement transducer (Grass FT-03).

Results and discussion. The isolated muscle strips were immersed in Tyrode solution containing DL-epinephrine or DL-norepinephrine at the concentration of 10^{-6} to 10^{-4} *M*[1,8-10] to allow the accumulation of catecholamine by $uptake_2$ process. 15 min after[9] they were washed out twice with amine-free medium to obtain the complete recovery of resting muscle tone and then exposed to ouabain (10^{-5} *M*). As shown in Figure 1 A, ouabain induced 2 steps of contraction; the rapid phase contraction and the late phase contraction. The rapid phase contraction starts immediately after exposure to ouabain, attains the peak level within 3 min and then returns to the resting level. About 10 min later, the late phase contraction developed, increased and reached the peak level after around 25 min. When the muscle strip was immersed in solution containing epinephrine rather than norepi-

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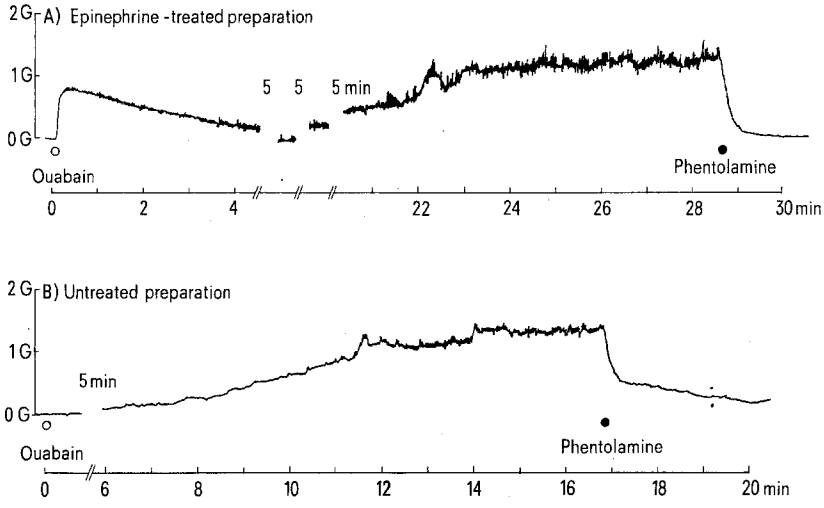

Fig. 1. Comparison of contractile pattern induced by ouabain (10^{-5} *M*) in the preparations of guinea-pig vas deferens pretreated with (A) and without (B) epinephrine. Preparation (A) was exposed to epinephrine (3×10^{-6} *M*) for 15 min, washed out with amine-free medium and then, 3 min later, immersed in the solution containing ouabain. Concentration of phentolamine is 10^{-5} *M*. Note the delayed initiation of late phase contraction in the preparation (A) in comparison to the preparation (B).

Effect of drugs to the maximum tension of the rapid contraction induced by ouabain[a]

Experimental procedures	Ouabain (10^{-5} *M*)-induced contractile tension (g)[d]	
Epinephrine (3×10^{-5} *M*), washout	1.77 ± 0.13	
Metanephrine (3×10^{-5} *M*)[c] epinephrine, washout	0.82 ± 0.07	($p < 0.01$)[e]
DOC (3×10^{-5} *M*)[c] epinephrine, washout	0.43 ± 0.17	($p < 0.001$)[e]
Oxytetracycline (10^{-4} *M*)[c] epinephrine, washout	2.11 ± 0.23	($p < 0.01$)[e]
Epinephrine, washout, phentolamine (10^{-5} *M*)[b]	0.14 ± 0.03	($p < 0.001$)[e]
Epinephrine, washout, bretylium (7×10^{-5} *M*)[b]	1.87 ± 0.14	(N.S.)

[a] Ouabain was added into the bath 10 min after washout with amine-free medium. [b] Phentolamine and bretylium were added immediately after washout. [c] Inhibitor of uptake$_2$ was used at adequate concentration without influence on a contractile response of 10^{-5} *M* epinephrine and introduced into the bath 5 min prior to the addition of epinephrine. [d] Values are given as mean $\pm$ SE of at least 6 preparations. [e] Each response differs significantly from the control response. N.S. means not significant.

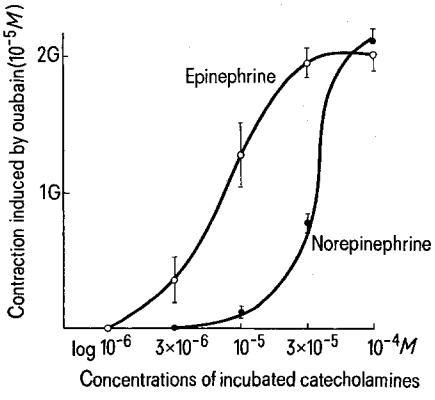

Fig. 2. Rapid phase contractions induced by ouabain after exposure to various concentrations of epinephrine and norepinephrine for 15 min. Ouabain (10^{-5} *M*) was added into the bath 3 min after washout of the preparation with amine-free medium. Vertical bars indicate standard errors. Each point is the mean of 6 preparations.

nephrine, a higher responsiveness of ouabain in the rapid phase was taken out (Figure 2). Moreover, epinephrine taken up by uptake$_2$ process was more resistant than norepinephrine to washing out with amine-free medium. These facts are similar to the results reported by IVERSEN[1] in the rat perfused heart. In the muscle strip which was not previously immersed in solution containing catecholamine, the late phase contraction alone was induced by ouabain (Figure 1 B). The late phase contraction was markedly inhibited by phentolamine (10^{-5} *M*), bretylium (7×10^{-5} *M*) and reserpine (1st day 2 mg/kg, 2nd and 3rd day 1 mg/kg, i.p.; $p < 0.001$ in all cases), but not by atropine or methysergide. These facts suggest that the late phase contraction results from the enhancement of the catecholamine-release from the neuronal site by ouabain[10,11]. On the other hand, the rapid phase contraction was scarcely effected by bretylium or reserpine, but was significantly prevented by pretreatment with DL-metanephrine (up to 3×10^{-5} *M*) or DOC (3×10^{-5} *M* dissolved in 0.5% ethanol), the specific inhibitors of uptake$_2$. Phentolamine, which was very effective in inhibiting the late phase (Figure 1), was the strong inhibitor to the rapid phase, too. POWIS[12] has stated that oxytetracycline prevented the uptake of catecholamine to collagen and elastic tissue. In this experiment, however, ouabain-induced rapid contraction was not prevented, but rather enhanced by oxytetracycline. These results were summarized in the Table.

The present findings provide evidence that a certain activity of Na–K ATPase is essential for the storage of catecholamine in extraneuronal site taken up by uptake$_2$ process and that the rapid phase contraction is due largely to the catecholamine released from the extraneuronal site by the ATPase-inhibition of ouabain.

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