Br. J. Pharmac. (1979), **65**, 473–478

ON THE MECHANISM OF PANCURONIUM-INDUCED SUPERSENSITIVITY TO NORADRENALINE IN RAT SMOOTH MUSCLE

D.R. TOMLINSON

Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Clifton Boulevard, Nottingham NG7 2UH

1 Pancuronium bromide (5×10^{-5} M) caused supersensitivity to noradrenaline in the rat isolated vas deferens and hepatic portal vein. This supersensitivity was manifest as a parallel leftward shift of the dose-response curve for noradrenaline with no alteration of the maximum response to the agonist.

2 Pancuronium did not potentiate the response of the vas and portal vein to St 91, an α-adrenoceptor agonist which is not a substrate for $Uptake_1$.

3 Pancuronium did not potentiate the response of the vas to $CaCl_2$.

4 In vasa deferentia made supersensitive to noradrenaline by treatment with cocaine (1×10^{-5} M) pancuronium induced no further potentiation of the response to noradrenaline. However, the supersensitivity to noradrenaline induced by pancuronium alone was augmented by the addition of cocaine.

5 Histofluorescence studies showed that pancuronium inhibited neuronal uptake of α-methyl noradrenaline in various noradrenergically-innervated tissues from reserpine-treated rats.

6 This study, with support from the literature, suggests that pancuronium induces noradrenaline supersensitivity by blockade of $Uptake_1$.

Introduction

Pancuronium, a widely-used competitive neuromuscular blocking drug, has been found by a number of workers to produce tachycardia and an increase in systemic arterial blood pressure in anaesthetized patients (see review by Walts, 1975) and in animals (Smith, Proctor & Spence, 1970).

This effect has been variously ascribed to blockade of muscarinic receptors leading to a reduction in vagal tone (Saxena & Bonta, 1970), blockade of neuronal uptake of noradrenaline (NA) (Ivankovich, Miletich, Albrecht & Zahed, 1975) and an indirect sympathomimetic effect (Domenech, Garcia, Sasiain, Loyola & Oroz, 1976). This study was designed to examine the second two postulates by investigating the action of pancuronium bromide on noradrenergically innervated tissues *in vitro*.

Birmingham & Hussain (personal communication) found that pancuronium (1×10^{-5} M to 2×10^{-5} M) potentiated the response of the guinea-pig isolated vas deferens to preganglionic electrical stimulation of its nerve supply. This finding prompted the present study in which the action of pancuronium at the noradrenergic neuroeffector junction in the isolated vas deferens and hepatic portal vein of the rat has been examined.

Methods

Male Wistar rats (weight range 200 to 250 g) were killed by a blow on the head and bled from the throat; tissues were then removed for experiments *in vitro*.

Rat vas deferens

The right vas deferens was removed from a freshly killed animal and suspended in a 20 ml organ bath containing Krebs solution (composition mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 30, NaH_2PO_4 1, and glucose 11.1) gassed with 95% O_2 and 5% CO_2 and maintained at 37°C. Contractions (longitudinal shortening) of the vas were recorded by means of an isotonic transducer (S.R.I.) driving a flat-bed recorder (Farnell). The recording system was calibrated before each experiment by stepwise linear dis-

0007-1188/79/030473-06 $01.00

placement of the transducer arm over the full range produced by contractions of the tissue. Displacement and pen excursion were linearly related; all contractions are expressed in units of pen excursion. The same apparatus was used for all preparations.

Log dose-response curves for noradrenaline bitartrate (Sigma) were obtained by adding the agonist to the bath (to achieve the concentrations shown in Figure 1) for a tissue contact time of 30 s. A dose of NA was given every 5 min. Dose-response curves were repeated until two consecutive dose ranges gave similar responses, at which point the bathing fluid was changed to Krebs solution containing 5×10^{-5} M pancuronium bromide (Organon) and the same dose ranges of NA were repeated to establish a reproducible dose-response relationship.

In a second series of experiments, control dose-response relationships for NA were again established. Following this, reproducible dose-response curves for NA were obtained in the presence of cocaine hydrochloride at a concentration of 1×10^{-5} M. Finally, pancuronium (5×10^{-5} M) was added to the bathing fluid as well as the cocaine and the NA dose-response relationship was redetermined.

In a third series of experiments the vasa were set up in the presence of pancuronium (5×10^{-5} M) and the NA dose-response relationship was established. Cocaine (1×10^{-5} M) was then added in the continued presence of pancuronium and the NA dose-response relationship was re-established.

The effect of pancuronium on the response of the vas to the agonist, St 91 (as the hydrochloride) was also examined. St 91 (Boehringer) is a clonidine derivative which is a potent α-adrenoceptor stimulant and which does not possess the structural requirements to make it a substrate for $Uptake_1$ in the noradrenergic terminal (Hoefke, Kobinger & Walland, 1975).

St 91 was used with a tissue contact time of 45 s and a 5 min cycle. Reproducible dose-response curves were obtained before and after the addition of pancuronium (5×10^{-5} M) to the bathing fluid.

The dose-response relationships for calcium ions (see Figure 2b) were obtained in a different manner. $CaCl_2$ has a limited solubility in Krebs solution so that Tyrode solution was used to achieve the necessary high Ca^{2+} concentrations. In addition the K^+ concentration was raised to increase smooth muscle excitability and facilitate the depolarizing action of Ca^{2+}. The bathing fluid had the following composition (mM): NaCl 100, KCl 40, $MgCl_2$ 1.1, $NaHCO_3$ 11.9, NaH_2PO_4 0.4 and glucose 5.9; $CaCl_2$ being omitted from the solution. The fluid was gassed with O_2 and maintained at 37°C. The vas was initially set up in the above solution with the addition of 1.1 mM disodium edetate (EDTA) and incubated for 30 min to remove Ca^{2+} from the extracellular fluid. The bathing fluid was then changed to one of the same composition, but without the EDTA, and $CaCl_2$ was added to increase progressively the bath concentration of Ca^{2+} ions (as shown in Figure 2). The tissue was allowed 15 min to contract maximally in each $CaCl_2$ concentration and cumulative dose-response curves were thus obtained. These were repeated until two consecutive dose ranges gave similar responses, then the bathing was fluid changed for one containing pancuronium bromide (5×10^{-5} M) and the $[Ca^{2+}]$-response relationship re-established.

In contrast to the studies involving NA, different preparations showed considerable variation in their maximum responses to $CaCl_2$. Therefore, to permit inter-animal comparison, the response of each preparation to each concentration of $CaCl_2$ (both in the presence and absence of pancuronium) has been expressed as a percentage of the maximum response to $CaCl_2$ in the absence of pancuronium.

Rat hepatic portal vein

The hepatic portal vein was removed from a freshly killed rat and suspended in an organ bath, containing Krebs solution, in a manner identical to that described for the vas. Reproducible log dose-response curves for NA were obtained before and after the addition of pancuronium (5×10^{-5} M) to the bathing fluid. Two responses to 1×10^{-6} M St 91 (approximately ED_{50}) were also obtained before and after pancuronium.

Effect of pancuronium on $Uptake_1$

Uptake of NA into noradrenergic nerve terminals ($Uptake_1$) was examined by a fluorescence histochemical method (see, for instance, Hamberger, 1967).

Rats were given a single intraperitoneal injection of reserpine ('Serpasil': CIBA) at a dose of 2 mg/kg. The rats were killed 18 h later and the irides, hepatic portal veins, abdominal venae cavae, right atria and portions of duodenal to ileal mesentery were removed and placed in Krebs solution gassed with 95% O_2 and 5% CO_2 at 37°C for 15 min. Some tissues were then incubated for a further 15 min in Krebs solution containing α-methyl noradrenaline hydrochloride ('Corbasil': Hoechst) at a concentration of 5×10^{-6} M. This was followed by two 5 min washes in Krebs solution to remove unbound α-methyl noradrenaline (α-methyl NA). All tissues were then treated with aqueous glyoxylic acid (Sigma) and made into whole mount preparations for fluorescence microscopy as described by Furness & Costa (1975). Other tissues served as controls and were incubated for 40 min in Krebs solution, without any exposure to α-methyl NA, before treatment with glyoxylic acid. For experiments in which inhibitors of $Uptake_1$ were studied

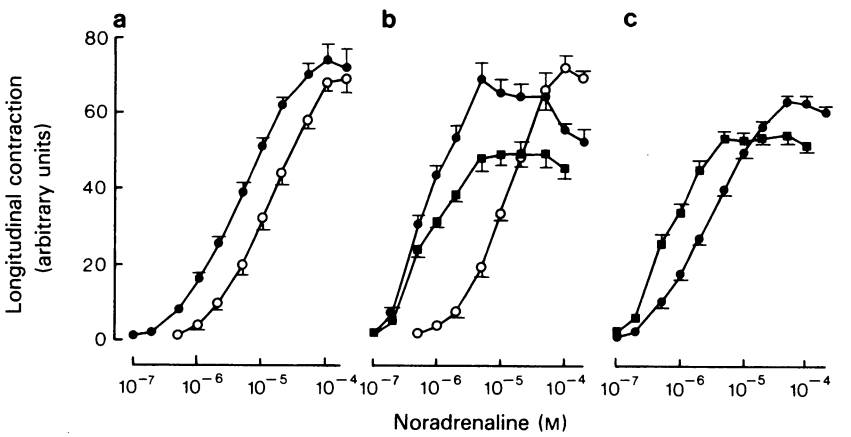

Figure 1 Log dose-response curves of rat isolated vasa deferentia to noradrenaline. (a) Before (○) and after (●) addition of pancuronium bromide (5×10^{-5} M) to the bathing fluid. (b) No drugs present (○), cocaine hydrochloride (1×10^{-5} M) added (●) and pancuronium bromide (5×10^{-5} M) added as well as cocaine (■). (c) Pancuronium bromide (5×10^{-5} M) present (●), later supplemented with cocaine hydrochloride (1×10^{-5} M) (■). Vertical lines denote s.e. mean; $n = 5$ rats for each of (a), (b) and (c). Response is expressed in units of pen excursion (see Methods).

(see Results) the putative inhibitor was present during the whole incubation *in vitro*, including the 15 min before the addition of α-methyl NA.

Results

Influence of pancuronium on the response of the vas to noradrenaline

Pancuronium alone, at concentrations of 5×10^{-5} M and 5×10^{-4} M, elicited no contractile activity from the vas deferens.

At 5×10^{-5} M pancuronium potentiated the response of the vas to NA. This was manifest as a parallel shift of the NA log dose-response curve to the left (Figure 1a). The increase in response to 1×10^{-5} M NA caused by pancuronium was highly significant ($P < 0.001$ by Student's paired t-test). There was no significant alteration in the maximum response to NA.

This effect was not enhanced at higher concentrations of pancuronium and was not seen at 1×10^{-5} M.

Interactions between pancuronium and cocaine

Cocaine has been demonstrated to cause supersensitivity to NA in noradrenergically innervated organs (findings reviewed by Iversen, 1967). This supersensitivity has been shown to be partly due to a blockade of Uptake$_1$ supplemented by an unexplained mechanism which generates a non-specific supersensitivity, evident on treatment with other agonists (Kasuya & Goto, 1968).

If pancuronium caused NA supersensitivity by a mechanism which differed from that of cocaine, then one might expect pancuronium to be capable of supplementing the supersensitivity produced by cocaine. This hypothesis was tested. Figure 1b shows that cocaine caused a greater leftward shift of the NA log dose-response curve than did pancuronium (Figure 1a). However, addition of pancuronium to the cocaine already present somewhat lessened the cocaine-induced supersensitivity to NA (Figure 1b).

Reversal of this procedure, that is treatment with cocaine of a vas previously made supersensitive to NA by pancuronium, showed that cocaine was capable of augmenting the effect of pancuronium (Figure 1c).

Influence of pancuronium on the response of the vas to St 91 and $CaCl_2$

Pancuronium (5×10^{-5} M) depressed the response of the vas to the α-adrenoceptor agonist, St 91 (Figure 2a). The reduction in the maximum response to St 91 was significant ($0.01 > P > 0.001$ by paired t test). This concentration of pancuronium also depressed slightly contractures of the vas induced by $CaCl_2$ (Figure 2b).

Influence of pancuronium on the response of the hepatic portal vein to noradrenaline and St 91

Pancuronium alone at concentrations of 5×10^{-5} M elicited no contractile response from the portal vein.

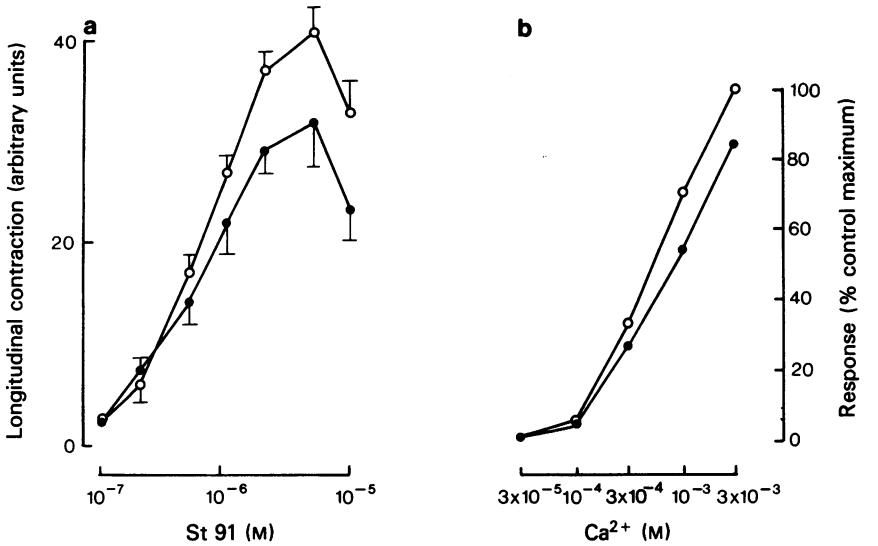

Figure 2 Log dose-response curves of rat isolated vasa deferentia to (a) St 91 and (b) $CaCl_2$ before (○) and after (●) addition of pancuronium bromide (5×10^{-5} M) to the bathing fluid. Vertical lines denote s.e. mean; $n = 5$ rats for both (a) and (b). Response is expressed in units of pen excursion (see Methods).

Indeed, at 5×10^{-4} M pancuronium inhibited the spontaneous contractions of the preparation.

The log dose-response curve for NA was shifted to the left by pancuronium at a concentration of 5×10^{-5} M. This shift was parallel and was associated with no change in the maximum response to NA. The increase in response to 1×10^{-6} M was significant ($0.01 > P > 0.001$ by paired t test) though not as great as that seen in the vas deferens (see Figure 3).

Pancuronium (5×10^{-5} M) depressed the response of the portal vein to 1×10^{-6} M St 91. This effect was statistically significant ($0.05 > P > 0.01$ by paired t test; $n = 5$).

Effect of pancuronium on α-methyl noradrenaline uptake by noradrenergic nerve terminals

Incubation of NA-depleted tissues from reserpine-treated rats with α-methyl NA (5×10^{-6} M) restores histofluorescence. This occurs because the α-methyl NA is accumulated by the nerves and the α-methylated amine, being resistant to monamine oxidase, remains in the axoplasm to form a fluophore. The uptake of α-methyl NA under these conditions is sensitive to drugs which block $Uptake_1$ (Hamberger, 1967).

This method of examining pharmacological interference with $Uptake_1$ suffers from the disadvantage that the degree of α-methyl NA uptake, and hence the degree of its impairment, cannot be quantified. However, the method does have the advantage that it examines only $Uptake_1$ and not an amalgam of this process and extraneuronal uptake (see Iversen, 1967).

The effect of pancuronium on the restitution of histofluorescence by α-methyl NA in a selection of noradrenergically innervated tissues from reserpine-treated rats (see Methods), was studied by incubation with pancuronium at concentrations of 5×10^{-5} M, 2×10^{-4} M, 5×10^{-4} M and 1×10^{-3} M. As a basis for comparison, desmethylimipramine (1×10^{-5} M) and chlorpromazine (1×10^{-5} M) were also used. These latter drugs, at the concentrations cited, were found (Hamberger, 1967) to inhibit completely the restitution of histofluorescence to reserpine-treated tissues on incubation with α-methyl NA. These findings were confirmed in the present study.

At the concentration which had produced the supersensitivity to NA in the vas and hepatic portal vein (5×10^{-5} M), pancuronium did not inhibit uptake of α-methyl NA to any perceptible extent. However, at higher concentrations pancuronium inhibited the restitution of histofluorescence by α-methyl NA (in all the tissues cited in the Methods section from four rats). This effect was marked at 2×10^{-4} M and complete at 5×10^{-4} M, indicating that the drug inhibited $Uptake_1$ at these concentrations.

Discussion

Pancuronium bromide, at 5×10^{-5} M, increased the sensitivity of rat isolated vas deferens and hepatic

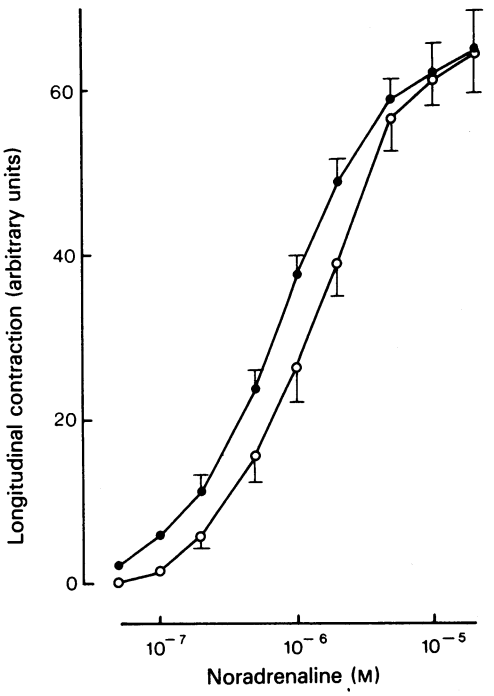

Figure 3 Log dose-response curves of rat isolated hepatic portal veins to noradrenaline before (○) and after (●) addition of pancuronium bromide (5×10^{-5} M) to the bathing fluid. Vertical lines denote s.e. mean; $n = 5$ rats. Response is expressed in units of pen excursion (see Methods).

portal vein to NA. Quintana (1977) obtained a similar result with the rat vas deferens. The latter study and the present investigation also demonstrated that pancuronium alone, at concentrations up to 5×10^{-4} M, elicited no contractile activity in the vas, a preparation which responds to indirect sympathomimetic amines. Thus the noradrenaline-releasing effect of pancuronium, seen in the dog *in vivo* by Domenech *et al.* (1976) was not evident in the rat tissues studied *in vitro*.

The observation that pancuronium did not potentiate the response of the vas or portal vein to St 91, an α-adrenoceptor stimulant which does not possess the structural requirements for $Uptake_1$ (Hoefke *et al.*, 1975; Ross, 1976), indicates that pancuronium might inhibit $Uptake_1$, thereby inducing NA supersensitivity. This suggestion is supported by the histofluorescence studies on α-methyl NA uptake. Further support comes from Ivankovich *et al.* (1975), who showed that pancuronium reduced the capture of isotopically labelled NA by the perfused rat heart. This finding does not distinguish between blockade of neuronal NA uptake ($Uptake_1$) and extraneuronal uptake. However, the finding that 2×10^{-4} M pancuronium caused a marked inhibition of neuronal α-methyl NA uptake correlates well with the demonstration that 1.36×10^{-4} M pancuronium produced a 50% inhibition of labelled NA uptake by the rat heart (Ivankovich *et al.*, 1975).

Clearly there is some disparity between these concentrations of pancuronium, which are required to produce a marked inhibition of catecholamine uptake, and the concentration which evokes maximal NA supersensitivity (5×10^{-5} M) in the vas and portal vein. Such disparity has also been found to exist with cocaine (Kalsner & Nickerson, 1969). It may be a reflection of the different time courses of the two events, namely the rapid build up of NA concentration at the receptor level following injection of the agonist into the organ bath and the more lengthy accumulation of amine by the nerve terminal over a period of incubation or perfusion.

Pancuronium did not augment the NA supersensitivity produced by cocaine in the vas. Conversely cocaine did augment the pancuronium-induced NA supersensitivity. This may be explained by the evidence which indicates that NA supersensivity to cocaine is due to an amalgam of $Uptake_1$ blockade and a non-specific supersensitivity to a number of agonists including calcium ions (Kasuya & Goto, 1968; Greenberg & Long, 1971). The present study shows clearly that pancuronium, unlike cocaine, does not enhance the sensitivity of the vas to $CaCl_2$.

Quintana (1977) suggested that the steroidal structure of pancuronium might confer some of the properties exhibited by the corticosteroids and some of their derivatives. These drugs promote an acute NA supersensitivity in cardiac and arterial smooth muscle, 17 β-oestradiol being the most potent (see, for instance Kalsner, 1969; Iversen & Salt, 1970). However, it is interesting that the response of the rat vas deferens to corticosteroids is atypical; 17 β-oestradiol is a potent non-competitive antagonist of NA on this preparation (Tomlinson unpublished). It is possible that pancuronium does exhibit weakly some properties of 17 β-oestradiol because this would explain the depression of the response of the vas to St 91 seen after pancuronium and the depression of the response to NA seen on administration of pancuronium after cocaine (see Figures 1b and 2a).

The NA supersensitivity of vascular smooth muscle caused by pancuronium is probably of greater significance to the anaesthetist than the extensively studied effects on the heart (Saxena & Bonta, 1970; Ivankovich *et al.*, 1975; Domenech *et al.*, 1976). Fuzzey & Edwards (1971) examined the effect of pancuronium on heart rate, arterial blood pressure and calf muscle blood flow in anaesthetized patients with peripheral vascular disease. Some patients exhibited tachycardia and hypertensive episodes, but the change in heart

rate for the group was not significant. However, calf muscle blood flow fell by a mean value of approximately 30% ($P < 0.02$).

In conclusion it can be stated that the findings of this study, taken together with those of Ivankovich *et al.* (1975), show that pancuronium bromide inhibits $Uptake_1$ for NA, thereby promoting a selective supersensitivity for this agonist in the isolated vas deferens and hepatic portal vein of the rat.

I would like to thank Mr Andrew Jackson for his excellent technical assistance. Thanks are also due to Organon Laboratories Ltd., Hoechst U.K. Ltd. and to C.H. Boehringer Sohn, Ingelheim for their gifts of drugs.

References

DOMENECH, J.S., GARCIA, R.C., SASIAIN, J.M.R., LOYOLA, A.Q. & OROZ, J.S. (1976). Pancuronium bromide: an indirect sympathomimetic agent. *Br. J. Anaesth.* **48,** 1143–1148.

FURNESS, J.B. & COSTA, M. (1975). The use of glyoxylic acid for the fluorescence histochemical demonstration of peripheral stores of noradrenaline and 5-hydroxytryptamine in whole mounts. *Histochem.*, **41,** 335–352.

FUZZEY, G.J.J. & EDWARDS, J.C. (1971). The change in calf muscle blood flow in the ischaemic limb during anaesthesia with pancuronium bromide. *Br. J. Anaesth.*, **43,** 753–758.

GREENBERG, S. & LONG, J.P. (1971). The effects of cocaine, norepinephrine and ionic stimulants on the isolated, superfused rat vas defens: antagonism by 'adrenergic neurone blockers' and reserpine. *J. Pharmac. exp. Ther.*, **177,** 136–145.

HAMBERGER, B. (1967). Reserpine-resistant uptake of catecholamines in isolated tissues of the rat. *Acta Physiol. scand.*, Suppl. **295,** 1–56.

HOEFKE, W., KOBINGER, W. & WALLAND, A. (1975). Relationship between activity and structure in derivatives of clonidine. *Arzneim-Forsch.*, **25,** 786–793.

IVANKOVICH, A.D., MILETICH, D.J., ALBRECHT, R.F. & ZAHED, B. (1975). The effect of pancuronium on myocardial contraction and catecholamine metabolism. *J. Pharm. Pharmac.*, **27,** 837–841.

IVERSEN, L.L. (1967). *The Uptake and Storage of Noradrenaline in Sympathetic Nerves.* Cambridge: University Press.

IVERSEN, L.L. & SALT, P.J. (1970). Inhibition of catecholamine $uptake_2$ by steroids in the isolated rat heart. *Br. J. Pharmac.*, **40,** 528–530.

KALSNER, S. (1969). Steroid potentiation of responses to sympathomimetic amines in aortic strips. *Br. J. Pharmac.*, **36,** 582–593.

KALSNER, S. & NICKERSON, M. (1969). Mechanism of cocaine potentiation of responses to amines. *Br. J. Pharmac.*, **35,** 428–439.

KASUYA, Y. & GOTO, K. (1968). The mechanism of supersensitivity to norepinephrine induced by cocaine in rat isolated vas deferens. *Eur. J. Pharmac.*, **4,** 355–362.

QUINTANA, A. (1977). Effect of pancuronium bromide on the adrenergic reactivity of the isolated rat vas deferens. *Eur. J. Pharmac.*, **46,** 275–277.

ROSS, S.B. (1976). Structural requirements for uptake into catecholamine neurones. In *'The Mechanism of Neuronal and Extraneuronal Transport of Catecholamines'*. ed. Paton, D.M. pp 67–93. New York: Raven Press.

SAXENA, P.R. & BONTA, I.L. (1970). Mechanism of selective cardiac vagolytic action of pancuronium bromide. Specific blockade of cardiac muscarinic receptors. *Eur. J. Pharmac.*, **11,** 332–341.

SMITH, G., PROCTOR, D.W. & SPENCE, A.A. (1970). A comparison of some cardiovascular effects of tubocurarine and pancuronium in dogs. *Br. J. Anaesth.*, **42,** 923–927.

WALTS, L.F. (1975). Complications of muscle relaxants. In *Muscle Relaxants.* ed. Katz, R.L. pp. 209–244. Amsterdam: North-Holland Publishing Co.

(Received July 14, 1978.
Revised September 5, 1978.)