INHIBITION OF NEURONAL UPTAKE OF NORADRENALINE IN THE ISOLATED PERFUSED RAT HEART BY PANCRUORNIUM AND ITS HOMOLOGUES, ORG. 6368, ORG. 7268 AND NC 45

P. J. SALT, P. K. BARNES AND C. M. CONWAY

SUMMARY

The cardiovascular effects of pancuronium may be caused partly by an interaction of this drug with the sympathetic nervous system. We examined one possible mechanism of interaction, the effect on the re-uptake processes for noradrenaline. Pancuronium and its closely related steroidal homologues, Org. 6368, Org. 7268 and NC 45, were studied at a high concentration (500 \( \mu \text{mol litre}^{-1} \)) for inhibition of the uptake of tritiated noradrenaline into neuronal sites (Uptake\(_1\)) and extraneuronal sites (Uptake\(_2\)) in the isolated perfused rat heart. All drugs tested caused almost total inhibition of Uptake\(_1\). The bis-quaternary steroids pancuronium and Org. 6368 were selective for Uptake\(_1\) inhibition, the mono-quaternary steroids Org. 7268 and NC 45 also produced significant inhibition of Uptake\(_1\). Uptake\(_1\) inhibition was investigated in detail using lesser concentrations of the compounds. All four steroids were found to cause a concentration-dependent inhibition of Uptake\(_1\). It seems likely, therefore, that inhibition of neuronal uptake of noradrenaline plays a significant role in the aetiology of the chronotropic actions of pancuronium in the rat.

Pancuronium bromide is a bis-quaternary steroid, which is a potent non-depolarizing antagonist of neuromuscular transmission (Buckett, Hewett and Savage, 1967). It possesses minimal ganglion-blocking and histamine-releasing properties (Buckett et al., 1968) and does not produce the hypotension seen with tubocurarine (Loh, 1970; Smith, Proctor and Spence, 1970; Nightingale and Bush, 1973).

Pancuronium can affect the cardiovascular system by several mechanisms. Vagolytic properties of pancuronium have been demonstrated in the cat (Hughes and Chappie, 1976), the dog and the guinea-pig (Saxena and Bonta, 1970). It can affect the uptake mechanisms for noradrenaline in the rat. This has been shown directly in the isolated perfused rat heart (Ivankovich et al., 1975), indirectly in the pithed rat (Docherty and McGrath, 1978), and in the isolated rat vas deferens (Quintana, 1977). Pancuronium also produces indirect sympathetic stimulation in the dog (Domenech et al., 1976).

This study was designed to investigate in detail the effect of pancuronium in the isolated perfused rat heart on the uptake of tritiated noradrenaline into neuronal sites, a process termed Uptake\(_1\) (Iversen, 1963) and to look for possible inhibition of extraneuronal uptake of tritiated noradrenaline which has been designated Uptake\(_2\) (Iversen, 1965). The effects produced by pancuronium were compared with those produced by its steroidal neuromuscular blocking homologues, NC 45, Org. 6368 and Org. 7268.

MATERIALS AND METHODS

Male albino Wistar rats weighing 150–250 g were killed by cervical dislocation and their hearts were removed and perfused by the Langendorff technique as previously described (Iversen, 1963). The perfusion medium was a Krebs–Henseleit solution to which was added EDTA disodium salt at a concentration of 10 mg litre\(^{-1}\) and ascorbic acid 20 mg litre\(^{-1}\) to reduce auto-oxidation of noradrenaline and glucose 1 g litre\(^{-1}\). The Krebs solution was gassed with 5% carbon dioxide in oxygen and maintained at 37 \(^\circ\)C. After perfusion for 1 min with amine-free solution the hearts were perfused with Krebs solution containing tritiated DL-noradrenaline (The Radiochemical Centre Amersham 13.0 Ci mmol\(^{-1}\)) diluted with non-radioactive DL-noradrenaline to a final concentration of 10 ng ml\(^{-1}\) and 50 nCi ml\(^{-1}\) for Uptake\(_1\) experiments and 5 \(\mu\)g ml\(^{-1}\) and 125 nCi ml\(^{-1}\) for Uptake\(_2\) experiments (Iversen, Salt and Wilson, 1972). All drugs tested were dissolved directly in this Krebs solution containing tritiated

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dl-noradrenaline immediately before each experiment. The four drugs investigated were initially tested for inhibition of Uptake_1 and Uptake_2 at concentrations of 500 μmol litre⁻¹. Subsequently, dose-response curves for Uptake_1 inhibition by the four compounds were obtained by using a range of drug concentrations because of the selectivity of action of pancuronium for Uptake_1. For Uptake_1 screening perfusion was continued with tritiated dl-noradrenaline for 10 min and in the case of Uptake_2 for 4 min. The hearts were then removed, blotted free of perfusion medium and homogenized in 1% EDTA 2 ml; 10 ml of acid ethanol was then added and the samples centrifuged at 3000 rev min⁻¹ for 10 min. The radioactivity in 1-ml aliquots of the supernatant was measured in a Packard Tri-Carb liquid scintillation spectrometer after adding Biofluor 15 ml (New England Nuclear) as scintillator. The tissue content of labelled noradrenaline plus metabolites was calculated after correcting for the presence of tritiated noradrenaline in the extracellular space and, in the case of Uptake_2, for the uptake of tritiated noradrenaline into sympathetic nerve terminals (Iversen and Salt, 1970). At each drug concentration six hearts were perfused. A log-probit plot of the results for Uptake_1 and Uptake_2 was made, and the line of best fit was obtained by the method of least squares. From these plots the concentration of drug producing 50% inhibition of each uptake process (IC₅₀) was calculated.

RESULTS

At a concentration of 500 μmol litre⁻¹, all four compounds produced almost complete inhibition of Uptake_1. Pancuronium appeared to be relatively selective for inhibition of Uptake_1. The monoquaternary steroids (Org. 7268 and NC 45, produced considerable inhibition of Uptake_2 (table I).

When tested at smaller concentrations the compounds produced a dose-dependent inhibition of Uptake_1 (fig. 1). Pancuronium was the most potent in this respect with IC₅₀ value (concentration of drug producing 50% inhibition of Uptake) of 16.1 μmol litre⁻¹; Org. 6368 was 2.4 times less potent than pancuronium, and the corresponding values for

**Table I. Inhibition of Uptake_1 and Uptake_2 by pancuronium and its homologues**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Uptake_1 (± SEM)</th>
<th>Uptake_2 (± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(drug concn 500 μmol litre⁻¹)</td>
<td>(drug concn 500 μmol litre⁻¹)</td>
</tr>
<tr>
<td>Pancuronium</td>
<td>94.1 ± 0.6</td>
<td>20.9 ± 8.3</td>
</tr>
<tr>
<td>Organon 6368</td>
<td>85.5 ± 0.7</td>
<td>37.4 ± 4.3</td>
</tr>
<tr>
<td>Organon NC 45</td>
<td>85.6 ± 0.6</td>
<td>57.0 ± 2.1</td>
</tr>
<tr>
<td>Organon 7268</td>
<td>74.9 ± 1.3</td>
<td>86.8 ± 1.4</td>
</tr>
</tbody>
</table>

**Table II. IC₅₀ values for Uptake_1 inhibition. The IC₅₀ values and slopes were determined from the graphical data illustrated in figure 1**

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC₅₀ (μmol litre⁻¹)</th>
<th>Gradient of dose response curve (probit-log units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancuronium</td>
<td>16.1</td>
<td>1.31</td>
</tr>
<tr>
<td>Organon 6368</td>
<td>38.8</td>
<td>1.20</td>
</tr>
<tr>
<td>Organon NC 45</td>
<td>67.6</td>
<td>1.24</td>
</tr>
<tr>
<td>Organon 7268</td>
<td>158.8</td>
<td>1.27</td>
</tr>
</tbody>
</table>
INHIBITION OF UPTAKE by PANCURONIUM AND HOMOLOGUES

NC 45 and Org. 7268 were 4.2 and 9.9 respectively (table II).

DISCUSSION

The actions of pancuronium on the cardiovascular system are complex and have been the subject of controversy. In the original evaluation of pancuronium in clinical practice (Baird and Reid, 1967) little effect was noted on arterial pressure and heart rate, and these findings were subsequently confirmed (McDowell and Clarke, 1969). Loh (1970), however, showed that pancuronium produced a significant transient increase in arterial pressure in patients undergoing cardiac surgery. This was confirmed by Kelman and Kennedy (1971), who also documented an increase in heart rate and cardiac output in patients undergoing general surgery. Nightingale and Bush (1973) obtained similar findings in children.

The cause of the cardiovascular side-effects appears to be complicated and multifactorial. Evidence for a vagolytic effect which might explain the tachycardia associated with pancuronium is well established from animal studies (Saxena and Bonta, 1970; Hughes and Chapple, 1976). Nana, Cordan and Domokos (1973) demonstrated an increase in plasma catecholamine concentrations, especially noradrenaline, after injection of pancuronium in man, and there is strong evidence for an interaction between pancuronium and the sympathetic nervous system in animal studies. Ivankovich and his co-workers (1975) showed that pancuronium inhibits uptake of noradrenaline to the isolated perfused rat heart, although their data do not distinguish between inhibition of Uptake\(_1\) and Uptake\(_2\). These workers used a concentration of noradrenaline (200 ng ml\(^{-1}\)) which has been found to saturate Uptake\(_1\) and with which Uptake\(_2\) accounts for a significant proportion of the observed uptake (Iversen, 1971). In our experiments Uptake\(_1\) inhibition was examined using a concentration of noradrenaline in perfusate (10 ng ml\(^{-1}\)) at which Uptake\(_1\) was the predominant inactivation process for the sympathetic transmitter. In screening for Uptake\(_2\) inhibition, a large concentration of noradrenaline (5 \(\mu\)g ml\(^{-1}\)) was used. At this concentration Uptake\(_1\) is saturated and Uptake\(_2\) is the predominant process for the uptake of noradrenaline. Steroids have been shown to be potent and selective inhibitors of Uptake\(_2\) (Salt, 1972) and in any examination of the effect of pancuronium on the uptake of noradrenaline, this should be excluded. The processes by which noradrenaline is inactivated in \textit{vivo} and the physiological effects of inhibition of the inactivation processes have been described previously (Salt, Barnes and Beswick, 1979). The results of the present study are in agreement with those of Ivankovich and others (1975). Our study shows that pancuronium is a selective inhibitor of Uptake\(_1\) and, despite its steroid nucleus, has a poor affinity for Uptake\(_2\) sites. The inhibition of Uptake\(_1\) is dose-dependent and the IC\(_{50}\) value (16.1 \(\mu\)mol litre\(^{-1}\)), determined from our dose-response curve, is of the same order of magnitude as that deduced from the data produced by Ivankovich and others (1975).

This study also confirms the work of Docherty and McGrath (1978), who found indirect evidence that pancuronium is an Uptake\(_1\) blocker in the pithed rat. These workers found no potentiation of the chronotropic response to isoprenaline, which is the substrate for Uptake\(_2\) and is not transported into Uptake\(_1\) sites (Callingham and Burgen, 1966).

Domenech and co-workers (1976), in experiments on dogs, obtained evidence that pancuronium may act indirectly by releasing noradrenaline from sympathetic nerve terminals. However, using the same preparation as ourselves (the isolated perfused rat heart), Ivankovich and others (1975) found no evidence for the release of noradrenaline from sympathetic nerve terminals by pancuronium. Quintana (1977), using the isolated rat vas deferens preparation, was able to show that pancuronium is devoid of indirect adrenergic activity, but he demonstrated an increased sensitivity in the preparation to noradrenaline explicable on the basis of uptake inhibition of the neurotransmitter by pancuronium. It is feasible that pancuronium acts on the sympathetic nerve terminals both by an indirect, tyramine-like mechanism, as well as by inhibition of Uptake\(_1\). Species difference may explain the apparent discrepancy in these findings.

The other bis-quaternary steroid tested, Org. 6368, also produced dose-dependent inhibition of Uptake\(_1\), although it was less active in this respect than pancuronium. The mono-quartenary steroids tested, NC 45 and Org. 7268, inhibited Uptake\(_2\) in a dose-dependent fashion, but to a lesser extent than did the bis-quaternary steroids.

In the screening experiments, at high concentration, the mono-quartenary compounds produced significant inhibition of Uptake\(_2\). There appeared to be an inverse relationship between the degree of blockade of Uptake\(_1\) and Uptake\(_2\) produced by the four drugs tested. Thus, the least active inhibitor of Uptake\(_1\), Org. 7268, produced the greatest inhibition.
of Uptake$_2$. The structure-activity relationship for inhibition of the two uptake processes may depend, therefore, on the number of charged groups attached to the steroid nucleus. This is in agreement with the findings of Salt (1972) that a wide range of biologically active uncharged steroids were selective for Uptake$_2$ inhibition and did not inhibit Uptake$_1$.

Of the compounds examined in this experiment pancuronium is established in clinical practice and NC 45 is undergoing clinical trials. Org. 6368 and Org. 7268 are not at present in clinical use and were included to see how homologues of pancuronium and NC 45 behaved. Pancuronium causes cardiac stimulation in the rat and this paper suggests this may in part be caused by inhibition of Uptake$_1$. The rat is resistant to the effect of pancuronium at the neuromuscular junction (Sugrue, Duff and McIndewar, 1975). In order to produce prolonged blockade of skeletal muscle twitching in the pithed rat, at least 1 mg kg$^{-1}$ must be administered (Docherty and McGrath, 1978). The concentration of pancuronium that produced 50% inhibition of Uptake$_1$ was 11.8 $\mu$g ml$^{-1}$. NC 45 is slightly less potent as a neuromuscular blocker in the cat than is pancuronium, and possesses a much wider margin between neuromuscular blocking doses and the doses which produce an increase in heart rate (Durant, 1978). Docherty and McGrath (1979) have shown in the pithed rat that NC 45 is 2.1 times less potent as a relaxant and 33 times less potent as a potentiator of cardiac sympathetic transmission. The concentration of NC 45 that produced 50% inhibition of Uptake$_1$ was 43.1 $\mu$g ml$^{-1}$, nearly four times the IC$_{50}$ of pancuronium. This offers an explanation of why NC 45 appears to be free of cardiovascular side-effects.

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REFERENCES


**INHIBITION DE LA CAPTATION NEURONALE DE NORADRENALINE DANS LE COEUR PERFUSE ISOLE D'UN RAT AU MOYEN DE PANCURONIUM ET DE SES HOMOLOGUES, ORG. 6368, ORG. 7268 ET NC 45**

RESUME

Les effets cardiovasculaires du pancuronium peuvent être le résultat partiel d'une interaction de cette substance sur le système nerveux sympathique. Nous avons examiné un mécanisme possible d'interaction, l'effet sur les processus de re-captation pour la noradrenaline. On a étudié une haute concentration (500 µmol litre⁻¹) de pancuronium et de ses homologues stéroïdiens très proches, Org. 6368, Org. 7268 et NC 45, du point de vue de l'inhibition de la captation de la noradrenaline tritée dans les sites neuronaux (C aptation) et les sites extraneuronaux (C aptation) d'un cœur perfusé isolé de rat. Toutes les substances testées provoquèrent une inhibition presque totale de la Captation. Les stéroïdes biquaternaires du pancuronium et l'Org. 6368 démontrèrent un caractère sélectif en ce qui concerne la Captation, les stéroïdes mono-quaternaires Org. 7268 et NC 45 provoquèrent également une inhibition significative de la Captation. On a fait une étude détaillée de l'inhibition de la Captation, en utilisant des concentrations moins fortes des composés. On a remarqué que les quatre stéroïdes provoquaient tous une inhibition de la Captation, qui dépendait de la concentration. Il est vraisemblable, par conséquent, que l'inhibition de la captation neuronal de la noradrenaline joue un rôle important dans l'etiologie des actions chronotropiques du pancuronium chez le rat.

**INHIBICION DE LA CAPTACION NEURAL DE NORADRENALINA EN EL CORAZON PERFUSADO AISLADO DEL RATON MEDIANTE PANCURONIO Y SUS HOMOLOGOS, ORG. 6368, ORG. 7268 Y NC 45**

SUMARIO

Los efectos cardiovasculares del pancuronio pueden ser parcialmente causados por una interacción de dicha substancia con el sistema nervioso simpático. Examinamos un posible mecanismo de interacción, el efecto sobre los procesos de re-captación de noradrenalina. Se procedió al estudio del pancuronio y de sus homólogos esteroides estrechamente relacionados, Org. 6368, Org. 7268 y NC 45 en una alta concentración (500 µmol litro⁻¹) para inhibir la captación de noradrenalina tritada dentro de los sitios neurales (Captación) y de los sitios extraneurales (Captación) en el corazón perfusado aislado del ratón. Todas las sustancias bajo prueba ocasionaron una inhibición casi total de la Captación. Los esteroides biquaternarios Pancuronio y Org. 6368 fueron selectivos para la inhibición de la Captación, los esteroides monoquaternarios Org. 7268 y NC 45 produjeron también una inhibición significativa de la Captación. Se realizó un estudio detallado de la inhibición de la Captación, mediante el uso de concentraciones menores de los compuestos. Se observó que los cuatro esteroides todos ocasionaban una inhibición de la Captación, que dependía de la concentración. Por lo tanto, es muy probable que la inhibición de la captación neuronal de noradrenalina juegue un papel importante en la etiología de las acciones cronotrópicas del pancuronio en los ratones.