On the question of subclassification of α-adrenoceptors

There is much evidence to support the existence of subclasses of β-adrenoceptors (Brittain, Jack & Ritchie, 1970; Furchgott, 1970; Triggle, 1971; Arnold, 1972). The question of α-adrenoceptor subclassification remains, however, controversial. For example, the isomer activity ratios for the noradrenaline enantiomers are similar in nine tissues, including rabbit aorta and spleen, that have been studied by Patil, Patel & Krell, (1971) and Patil, Fudge & Jacobowitz (1972). Similarly, dissociation constants for phentolamine are identical in eight of nine tissues studied in different laboratories (Furchgott, 1970; Clineschmidt, Geller & others, 1970; Patil & others, 1972). However, Sheys & Green (1972) recently proposed the non-identity of the α-adrenoceptors in rabbit spleen and aorta on the basis that the apparent dissociation constants for several agonists and antagonists were different in these two tissues. In the rat and guinea-pig vasa deferentia we have found (Avner & Triggle, 1972) prosympal (2-diethylaminomethyl-1,4-benzodioxan, F-883) to be an active antagonist in the former but inactive in the latter preparation. Hence, the question remains unresolved.

We have suggested recently that the kinetics of recovery of response from irreversible α-adrenoceptor antagonism by short acting 2-halogenoethylamines may be sensitive to subtle differences at or near the receptor. The values obtained for the recovery of response from DMPEA (*NN*-dimethyl-2-bromo-2-phenylethylamine) for rat and rabbit aorta and rat vas deferens suggested that these receptors were different from those of the guinea-pig and rabbit vas deferens (Janis & Triggle, 1971a, 1972).

Results indicating that many 2-halogenoethylamines have at least two sites of interaction where they modify α-adrenoceptor response in rat vas deferens (Moran, Swamy & Triggle, 1970; Swamy & Triggle, 1972) and rat and rabbit aorta (Janis & Triggle, 1971b, 1972) have been reported. One of these sites, that at which the 2-halogenoethylamines exert a blockade of prolonged duration, is believed to be associated with a Ca^{2+} mobilizing function since a number of Ca^{2+} competing species, including local anaesthetics and diazoxide, convert the prolonged blockade to one of relatively short duration *without* affecting the degree of blockade. We have postulated that the site so protected is involved in membranal Ca^{2+} mobilization and may be allosterically linked to the noradrenaline recognition site so that the two sites together constitute the functional α-adrenoceptor (Moran & others, 1970; Triggle, 1971, 1972).

The possibility, therefore, exists that α-adrenoceptors may differ not only in the recognition site but also in this proposed associated Ca^{2+} binding site. To test this hypothesis we have determined whether cinchocaine, tetracaine and diazoxide can similarly modify the prolonged duration of action of SY-28 (*N*-(2-bromoethyl)-*N*-ethyl-1-naphthylmethylamine) in the rat and rabbit aorta and rabbit vas deferens. Because DMPEA has a relatively long duration of action ($t_{\frac{1}{2}} = 124$ min, Janis & Triggle, 1971a) in the latter tissue it was also feasible to include this agent in this study. The basic experimental procedure was that employed previously (Janis & Triggle, 1971b; Swamy & Triggle, 1972): SY-28 or DMPEA was used in concentrations that produced 75–100% inhibition of response to a maximum concentration of noradrenaline (10^{-4}M) and the extent of recovery of response to this concentration of noradrenaline determined at various time intervals. The $t_{\frac{1}{2}}$ for recovery of response of DMPEA-treated rabbit vas deferens agreed with that reported previously (120 ± 12 min) and this was not changed by the tetracaine or diazoxide pretreatment. Similarly, the prolonged duration of action of SY-28 in these three tissues ($t_{\frac{1}{2}} > 300$ min) was not significantly altered by the pretreatment agents (Table 1).

The contrast of these results to those previously obtained with the rat vas deferens (Moran & others, 1970; Swamy & Triggle, 1972) is quite striking and is suggestive

Table 1. *Effects of various treatments on the duration of α-adrenoceptor inhibition by* N-*(2-bromoethyl)*-N-*ethyl*-N-*naphthylmethylmethylamine* (SY-28) *and* NN-*dimethyl-2-bromo-2-phenylethylamine (DMPEA)*[a].

Irrev. Antag.	Pretreatment	Tissue: Aorta Rat	Aorta Rabbit	Vas deferens Rat	Vas deferens Rabbit
DMPEA	Tetracaine, 10^{-4}M				—
DMPEA	Diazoxide, 10^{-4}M				—
SY-28	Cinchocaine, 0·01–5 × 10^{-4}M ..	—	—	+	—
SY-28	Tetracaine, 1–5 × 10^{-4}M	—	—	+	
SY-28	Diazoxide, 0·5–1·0 × 10^{-4}M ..	—	—	+	

[a] Experiments in which treatment did or did not significantly decrease the rate of recovery are denoted by + and — respectively. The long acting agents were used at concentrations that produced 75–100% inhibition of response. (8×10^{-7}M SY-28 per 5 min for rabbit and rat aorta and 10^{-6}M SY-28 per 5 min for rabbit vas; 2×10^{-5}M DMPEA per 5 min for rabbit vas). The reversible pretreatment drug was added to the tissue 5 min. before the irreversible agent except for diazoxide which was added to Ca^{2+} free solution 10 min before addition: the latter protocol was adopted since it was that which was most successful for the rat vas deferens preparation (Swamy & Triggle, 1972). A minimum of three experiments were performed with each schedule. Tissue responses were recorded isometrically (vascular) and isotonically (nonvascular) as previously described (Janis & Triggle, 1971a, b, 1972). The data for the rat vas deferens are taken from Moran & others (1970) and Swamy & Triggle (1972).

of differences in α-adrenoceptor organization. It may be that the Ca^{2+} mobilization site proposed as a component of the α-adrenoceptor in the rat vas deferens is quite different or non-existent in the other three tissues that we report here. It is perhaps relevant in this connection that we have found (R. A. Janis & D. J. Triggle, unpublished data) that the rate of loss of response to noradrenaline of rat and rabbit aorta and rabbit vas deferens in Ca^{2+}-free media is substantially slower than for rat vas deferens.

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