Table 1 Comparison of different quantitative histochemical methods for the assessment of cytochrome oxidase activities in tissues from control rabbits and those receiving lethal doses of KCN (8 mg CN/kg, i.m.); results expressed as mean ± s.e.mean for 6 animals per group

Group	*Cytochrome oxidase activity measured by*			
	Kinetic microdensitometry (ΔOD)		*End-point histophotometry (OD)*	
	Myocardium	*Brain*	*Myocardium*	*Brain*
Control	0.060 ± 0.001	0.016 ± 0.001	0.46 ± 0.04	0.20 ± 0.03
Cyanide	0.033 ± 0.001	0.008 ± 0.001	0.31 ± 0.01	0.16 ± 0.01
*P**	0.001	0.001	0.001	0.001

* Significance of differences in enzyme activities between controls and cyanide injected animals (*t*-test).

amine (5 mg) and *p*-amino *p*-methoxydiphenylamine in 0.2 ml methanol. Fluid was poured over cover slips to a thickness of 1 mm and allowed to solidify. Cryostat sections of fresh-frozen tissues were applied to the gel films, immediately after which azine dye production was determined microdensitometrically by measuring the rate of change in optical density (ΔOD) with a Leitz MPV 2 microscope recording photometer equipped with a grating monochromator. Optimum results were obtained with 10 μm thick sections of 2–4 mm diameter and measurement of ΔOD at 550 nm.

Measurement of cytochrome oxidase activity by kinetic microdensitometry was compared with assessment of enzyme activity by conventional end-point histophotometry, in which tissue sections were incubated in histochemical substrate solutions (Burstone, 1960) and dye production estimated by a single measurement of optical density after 20 min incubation. Measurements were made on brain and myocardium from control (carbon dioxide euthanasia) and cyanide sacrificed rabbits (8 mg CN/kg, i.m.). Cytochrome oxidase activity (Table 1) was significantly lower in tissues from cyanide animals compared with controls, and measured enzyme inhibition was greater by kinetic microdensitometry (brain 50%; myocardium 45%) than by end-point histophotometry (brain 20%; myocardium 33%). With the latter approach spontaneous reactivation of the enzyme causes a progressively increased rate of substrate utilization, but using kinetic microdensitometry the initial reaction rate is measured before significant reactivation occurs.

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Profiles of α-adrenoceptor antagonists in the pithed rat

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There is evidence that presynaptic and postsynaptic α-adrenoceptors (Langer, 1974) differ in their sensitivity to antagonists (Starke, Borowski & Endo, 1975; Drew, 1976; Doxey, Smith & Walker, 1977). The profiles of 3 antagonists (yohimbine, phentolamine and prazosin) on pre- and post-synaptic α-adrenoceptors were studied in pithed rats. The ability of the 3 antagonists to inhibit electrically induced contractions of the vas deferens and anococcygeus muscle was used as an assessment of postsynaptic activity. Presynaptic activity was assessed by determining the ability of the antagonists to reverse the inhibitory effects of clonidine on sympathetic outflow to the vas deferens and anococcygeus muscle (Doxey & Everitt, 1977).

All studies were performed in pithed male CFY rats given tubocurarine (1 mg/kg, i.v.). Stimulation (6 Hz, 50 μs, 20–40 v for 3 s and 30 s alternately every 2 min) of the spinal sympathetic outflow supplying the vas deferens produced constant contractile responses of the tissue. A stimulus of 3 s duration produced a 'twitch' response; with a stimulus of 30 s duration the 'twitch' preceded a slow secondary contraction. In 3 rats phentolamine in doses up to 1 mg/kg, i.v. had no significant effect on contractions of the vas deferens. Yohimbine (1 mg/kg, i.v.) had no significant effect on the 'twitch' response but potentiated ($61 \pm 20\%$, $n = 3$) the slow contraction. Prazosin (1 mg/kg, i.v.) inhibited both the 'twitch' response ($29 \pm 5\%$, $n = 4$) and the slow contractile response ($45 \pm 5\%$, $n = 4$).

Contractions of the vas deferens (6 Hz, 50 μs, 20–40 v for 3 s every 30 s) were inhibited by clonidine (30 μg/kg, i.v.). Both yohimbine (0.1–0.3 mg/kg, i.v., $n = 5$) and phentolamine (0.03–0.1 mg/kg, i.v., $n = 7$) reversed the effects of clonidine. Prazosin (4 rats) in doses up to 1 mg/kg, i.v. had no effect on the inhibition produced by clonidine.

Contractions of the anococcygeus muscle (1 Hz, 40 v, 0.5 ms, 20 s every 1 min) were inhibited by both prazosin ($ED_{50} = 6 \pm 2$ μg/kg, i.v., $n = 4$) and phentolamine ($ED_{50} = 0.4 \pm 0.03$ mg/kg, i.v., $n = 4$) but yohimbine in 7 rats had no effect in doses up to 1 mg/kg, i.v. In 4 rats contractions of the anococcygeus muscle induced by noradrenaline (3 μg/kg, i.v.) were abolished by this dose of yohimbine. Clonidine also inhibited electrically induced contractions of the anococcygeus muscle ($ED_{50} = 1 \pm 0.2$ μg/kg, i.v., $n = 5$). The inhibition produced by clonidine was reversed by yohimbine (0.3–1.0 mg/kg, i.v., $n = 5$).

It has been reported that yohimbine acts preferentially at presynaptic α-adrenoceptors (Starke *et al.*, 1975; Drew, 1976; Doxey *et al.*, 1977), phentolamine is much less selective for presynaptic α-adrenoceptors than yohimbine (Drew, 1976; Doxey *et al.*, 1977) and in the rat prazosin acts selectively at postsynaptic α-adrenoceptors (Doxey *et al.*, 1977; Cavero, Lefèvre & Roach, 1977).

On the vas deferens presynaptic α-adrenoceptor antagonism of clonidine could only be demonstrated with yohimbine and phentolamine; prazosin appeared to be devoid of such activity at the doses studied. As in previous studies (Ambache & Aboo Zar, 1971) demonstration of postsynaptic α-adrenoceptor antagonist activity as judged by inhibition of electrically induced contractions of the vas deferens proved difficult even with a selective postsynaptic α-adrenoceptor antagonist such as prazosin. Using the anococcygeus muscle, however, both prazosin and phentolamine were effective postsynaptic α-adrenoceptor antagonists and abolished electrically induced contractions of the tissue. The finding that yohimbine would not inhibit electrically induced contractions of the anococcygeus muscle at doses which abolished contractions produced by exogenously applied noradrenaline reflects its high selectivity for presynaptic α-adrenoceptors. This was supported by the finding that yohimbine would reverse the inhibitory effects of clonidine on the anococcygeus muscle.

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