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THE EVALUATION OF THE NOVEL PRESSOR ACTIVITY OF γ-PIPERIDINOBUTYRAMIDE (WY 20051, DF480)

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1 γ-Piperidinobutyramide (Wy 20051, DF480) injected intravenously evoked pressor responses in the anaesthetized ganglion blocked rat preparation over the dose range 2.4×10^{-6}–3.0×10^{-4} mol/kg.

2 High doses ($>3.8 \times 10^{-5}$ mol/kg) or even repeated submaximal doses (1.9×10^{-5} mol/kg) of Wy 20051 caused tachyphylaxis of this pressor response.

3 The noradrenaline pressor-response curve was shifted significantly to the right of the control curve following a dose of Wy 20051 (1.5×10^{-4} mol/kg cumulative).

4 The dose-response curve for the pressor action of Wy 20051 was potentiated in reserpine-treated anaesthetized rats. In contrast, tyramine-induced pressor responses were abolished.

5 Wy 20051 contracted the guinea-pig isolated aortic spiral preparation (3.8×10^{-5}–6.0×10^{-4} mol) and evoked constrictor responses in the perfused mesenteric vasculature preparation of the rat (5.9×10^{-7}–1.2×10^{-5} mol). At higher doses the responses were reduced.

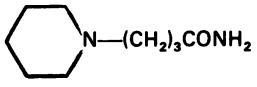
6 Wy 20051-induced constrictor responses of the perfused mesentery were unaffected by blockade of α-adrenoceptors or by tachyphylaxis of 5-hydroxytryptamine receptors.

7 The time for abolition of Wy 20051-induced constrictor responses of the mesentery in a calcium-free medium was not significantly different from that required for noradrenaline, but was significantly greater than that for KCl ($P < 0.001$).

8 Wy 20051 and noradrenaline, but not KCl, evoked constrictor responses in the depolarized rat mesenteric vasculature.

9 The results indicate that Wy 20051 evokes pressor responses which have some of the characteristics of those of noradrenaline. However, the responses are not elicited by an α-adrenoceptor mechanism.

Introduction



γ-Piperidinobutyramide (Wy 20051, DF480) a close derivative of γ-aminobutyric acid (GABA) was originally synthesized for comparative studies with the parent compound on the central nervous system. During examination of the pharmacology of Wy 20051 in the anaesthetized rat, it was observed that intravenous administration evoked transient pressor responses. The experiments described in this paper were designed to investigate the mode and site of action of this unusual pressor activity.

Methods

Intact animal studies

Female Charles River rats weighing 200–250 g were anaesthetized with pentobarbitone sodium (2.3×10^{-4} mol/kg i.p.). Each animal was allowed to breathe spontaneously through a tracheostomy tube. Drug-induced changes in blood pressure and heart rate were monitored by means of a Statham P23 pressure transducer from a catheter inserted into the left carotid artery and recorded on a Grass model 7 polygraph. Drugs were administered via a catheter into the jugular vein.

Ganglion blocked anaesthetized rats

A state of ganglion blockade was induced in each rat by administering pentolinium (2.0×10^{-5} mol/kg). The blockade was challenged with nicotine (6.2×10^{-7} mol/kg) 15 min after the administration of pentolinium and at intervals during the experiment. Further doses of pentolinium (8.4×10^{-6} mol/kg) were administered as required to maintain the blockade. A steady basal blood pressure was therefore established to facilitate measurement of pressor responses.

Dose-response curves to Wy 20051 (2.4×10^{-6}–3.0×10^{-4} mol/kg), noradrenaline (1.4×10^{-11}–1.5×10^{-8} mol/kg) or tyramine (1.8×10^{-8}–4.7×10^{-6} mol/kg) were obtained, followed by constant submaximal doses of each agonist (9.4×10^{-6} mol/kg, 2.4×10^{-9} mol/kg and 2.9×10^{-7} mol/kg respectively). In some experiments, dose-response curves to noradrenaline were obtained before and after a dose of Wy 20051 (1.5×10^{-4} mol/kg cumulative). In other experiments a constant submaximal dose of Wy 20051 (1.9×10^{-5} mol/kg) was administered every 7 min to anaesthetized rats and the effect on blood pressure recorded.

Reserpine-treated anaesthetized rats

Reserpine (8.2×10^{-6} mol/kg i.p.) was administered to rats 24 h before the start of the experiments. Only those animals in which tyramine (1.8×10^{-8}–4.7×10^{-6} mol/kg) did not evoke pressor responses were used. A dose-response curve to Wy 20051 (1.5×10^{-7}–3.8×10^{-5} mol/kg) was then obtained in the reserpine-treated rats.

Isolated tissue studies

All isolated tissues were suspended in (or perfused with) Krebs solution of the following composition (mmol/litre): NaCl 118.4 (13.0), KCl 4.7 (110.0), $NaHCO_3$ 25.0, KH_2PO_4 1.2, $CaCl_2$ 2.5 (0.25), $MgSO_4$ 1.2, glucose 11.0. The figures in parentheses refer to a depolarizing solution used in certain experiments. When a calcium-free Krebs solution was used $CaCl_2$ was omitted and 5.0×10^{-4} mol/litre ethyleneglycol bis-(aminoethyl)-tetracetic acid was added as a calcium chelating agent.

Where necessary, ascorbic acid (1.0×10^{-4} mol/litre) was added to the bathing fluid to prevent oxidation of noradrenaline. Organ bath volume was 35 ml. Perfusion rate for the perfused mesenteric vasculature preparation was 2 ml/minute.

Responses were recorded by variable inductance transducers connected to a phase discriminator and pen recorder or, in the case of the mesenteric vasculature preparation, pressure changes were recorded via a Bell and Howell L221-2-3 pressure transducer on a Devices M2 pen recorder.

The tissues were left to equilibrate for 1 h before dosing was started.

Guinea-pig aortic spiral

Contractions of the guinea-pig aortic spiral (Furchgott & Bhadrakom, 1953) were recorded (load 0.5 g) in response to doses of noradrenaline (1.1×10^{-7} mol) given at 5 min intervals until constant responses were obtained. The responses to Wy 20051 (3.8×10^{-5}–1.2×10^{-3} mol cumulative) or noradrenaline (1.2×10^{-9}–1.5×10^{-6} mol cumulative) were then observed.

Rat perfused mesenteric vasculature

The rat isolated perfused mesenteric vasculature preparation was set up as described by Collis & Alps (1973). Agonists were administered every 2 min or on restoration of basal perfusion pressure, whichever was the sooner. Constrictor responses were recorded as described.

Constrictor action of Wy 20051. Dose-response curves to noradrenaline (5.9×10^{-11}–4.7×10^{-8} mol) and constant responses to a submaximal dose (2.4×10^{-9} mol) were obtained. A response curve was then obtained to Wy 20051 (5.9×10^{-7}–1.2×10^{-4} mol).

Blockade of mesenteric constrictor responses. Approximately equal submaximal constrictor responses were obtained every 2 min with noradrenaline (1.2×10^{-9} mol), 5-hydroxytryptamine (5-HT, 4.5×10^{-9} mol), KCl (2.2×10^{-5} mol), $BaCl_2$ (9.6×10^{-6} mol) and Wy 20051 (4.7×10^{-6} mol). The tissue was then perfused with either (i) phenoxybenzamine (1×10^{-5} mol/litre); (ii) 5-HT (2.8×10^{-5} mol/litre); (iii) calcium-free Krebs solution or (iv) the depolarizing solution and the responses repeated. In some experiments with calcium-free Krebs solution the time taken for abolition of the mesenteric constrictor responses to noradrenaline, KCl and Wy 20051 was determined.

Effect of γ-aminobutyric acid. Doses of GABA (1.9×10^{-7}–1.9×10^{-4} mol) were given and the effect recorded.

α-Adrenoceptor blocking activity. Approximately equal submaximal constant responses were obtained to noradrenaline (4.7×10^{-9} mol) and KCl (1.1×10^{-5} mol). Bolus doses of Wy 20051 (4.7×10^{-5} mol) or phenoxybenzamine (3.3×10^{-10} mol) were given and 10 min later the responses to noradrenaline and KCl were repeated.

Drugs

Drugs used were (−)-ascorbic acid (BDH), ethyleneglycol bis-(aminoethyl)-tetracetic acid (Koch Light), 5-hydroxytryptamine creatinine sulphate (Koch Light), (−)-noradrenaline bitartrate (Koch Light), pentolinium bitartrate (May and Baker), phenoxybenzamine hydrochloride (Smith, Kline and French), reserpine (Ciba-Geigy) and tyramine hydrochloride (Koch Light). Drugs used in whole animal preparations were suspended in a 0.5% hydroxymethylcellulose-0.9% w/v NaCl solution

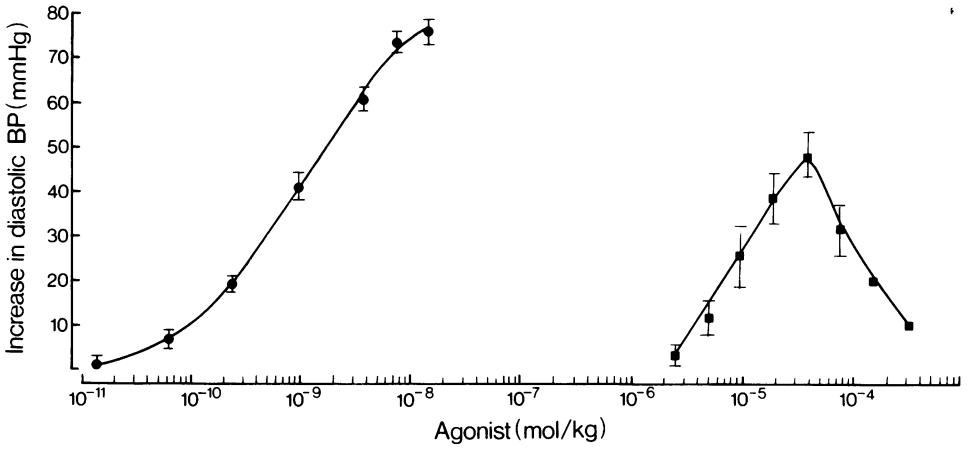


Figure 1 The effect of noradrenaline (●) and Wy 20051 (■) on the diastolic blood pressure of the anaesthetized ganglion blocked rat. Drugs were administered cumulatively i.v. via a cannula in the jugular vein. In contrast to noradrenaline high doses of Wy 20051 produced a diminished pressor response. Wy 20051 ($n=6$) was 1.28×10^4 times less potent than noradrenaline ($n=6$) when doses producing a 30 mmHg pressor response were compared.

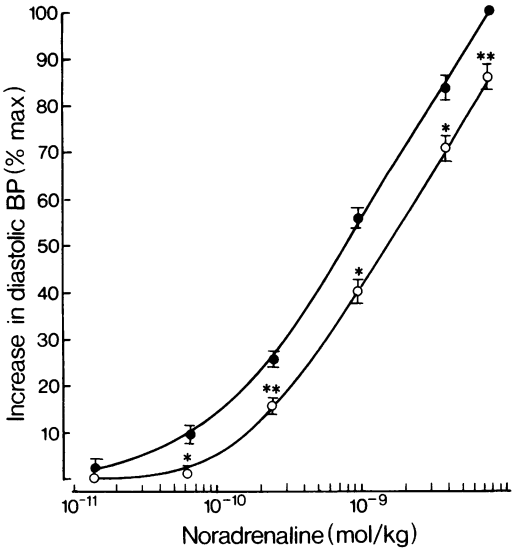


Figure 2 Response curves to noradrenaline before (●) and after (○) a cumulative dose of Wy 20051 (1.5×10^{-4} mol/kg ≙ 3.4×10^{-5} mol) in anaesthetized ganglion blocked rats ($n=12$). Note that after Wy 20051 administration, the noradrenaline dose-response curve was significantly shifted to the right of the control curve. ★ $P<0.01$; ★★ $P<0.001$.

vehicle. Agonists used in isolated tissue preparations were dissolved in either Krebs solution (aorta) or distilled water (mesentery).

Results

Ganglion-blocked anaesthetized rats

Effect on diastolic blood pressure and heart rate. Wy 20051 transiently increased diastolic pressure over the cumulative dose range 2.4×10^{-6}–3.0×10^{-4} mol/kg. At higher doses ($>3.8 \times 10^{-5}$ mol/kg) these pressor responses were diminished (Figure 1). The maximum response to Wy 20051 (43.0 ± 5.8 mmHg; $n=6$) was significantly less than that to noradrenaline (75.0 ± 2.5 mmHg; $n=6$) ($P<0.001$). Doses of 9.4×10^{-6} mol/kg that had elicited pressor responses approximating to ED_{30} before the response curve were ineffective when given after the response curve. At doses eliciting pressor responses, Wy 20051 had no effect on heart rate.

Effect of continual dosing. Continual administration every 7 min of a dose of Wy 20051 approximating to ED_{50} (1.9×10^{-5} mol/kg) caused tachyphylaxis of the pressor responses. Responses were reduced to 42% of the starting level after the fourth dose and to 11% after the tenth dose ($n=6$).

Effect of Wy 20051 on noradrenaline dose-response curves. The noradrenaline dose-response curve was shifted significantly to the right of the control curve following a cumulative dose of Wy 20051 (1.5×10^{-4} mol/kg ≙ 3.4×10^{-5} mol; Figure 2). The mean dose-ratio calculated at doses approximating to 30, 50 and 70% of the maximum response was 2.0.

Effect of tyramine on diastolic pressure. Tyramine produced a pressor effect within the dose range

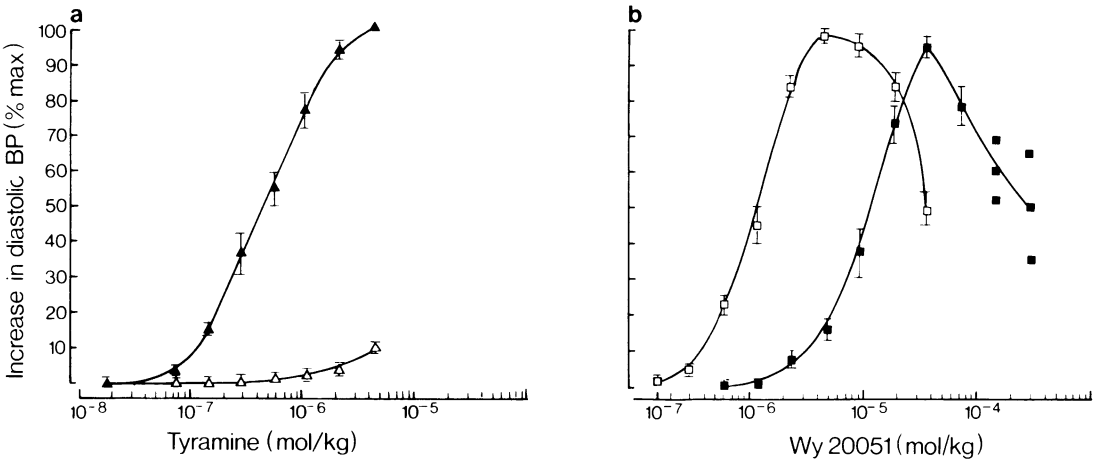


Figure 3 Effect of (a) tyramine and (b) Wy 20051 on the diastolic pressure of ganglion blocked (▲ and ■) and reserpine-treated (△ and □) rats. Rats were treated with reserpine or ganglion blocked as described in the text and tyramine ($n=6$) or Wy 20051 ($n=6$) given intravenously via a cannula in the jugular vein. Reserpine blocked tyramine-induced responses, but responses to Wy 20051 were potentiated.

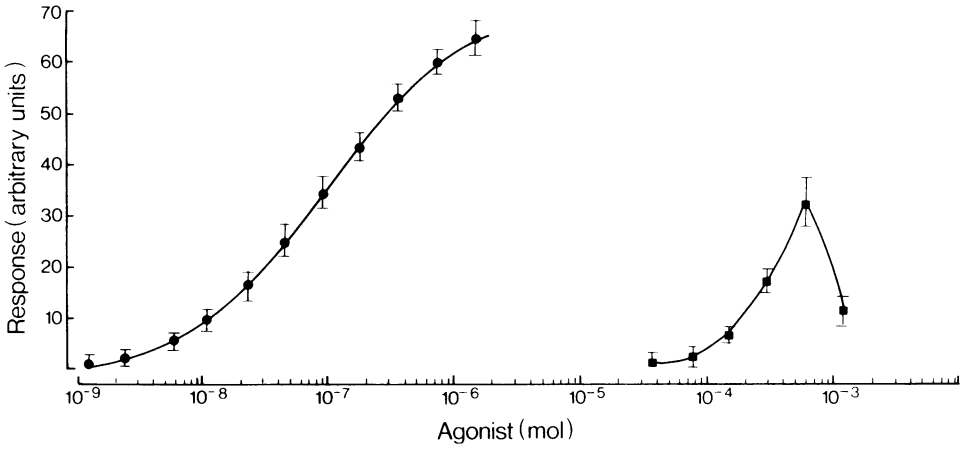


Figure 4 The constrictor activity of noradrenaline (●) and Wy 20051 (■) on the guinea-pig isolated aortic spiral preparation. The tissues were set up as described in the text. In contrast to noradrenaline high doses of Wy 20051 produced a diminished response. At the dose level producing a contraction of 30 arbitrary units, Wy 20051 ($n=5$) was 0.84×10^4 times less potent than noradrenaline ($n=5$).

1.8×10^{-8}–4.7×10^{-6} mol/kg (Figure 3a). Unlike Wy 20051, a constant submaximal dose of tyramine evoked equieffective pressor responses when administered after a dose-response curve and no diminution of responses was observed at high dose levels.

Reserpine-treated anaesthetized rats

The responses to tyramine previously obtained within the dose range 1.8×10^{-8}–4.7×10^{-6} mol/kg were not evoked in rats pretreated with reserpine (Figure 3a). In contrast, responses to Wy 20051 were potentiated in reserpine-treated animals (Figure 3b).

Guinea-pig aortic spiral

Wy 20051 (3.8×10^{-5}–6.0×10^{-4} mol cumulative) and noradrenaline (1.2×10^{-9}–1.5×10^{-6} mol cumulative) evoked contractions of the guinea-pig isolated aortic spiral. At higher doses of Wy 20051 (1.2×10^{-3} mol) the responses diminished (Figure 4).

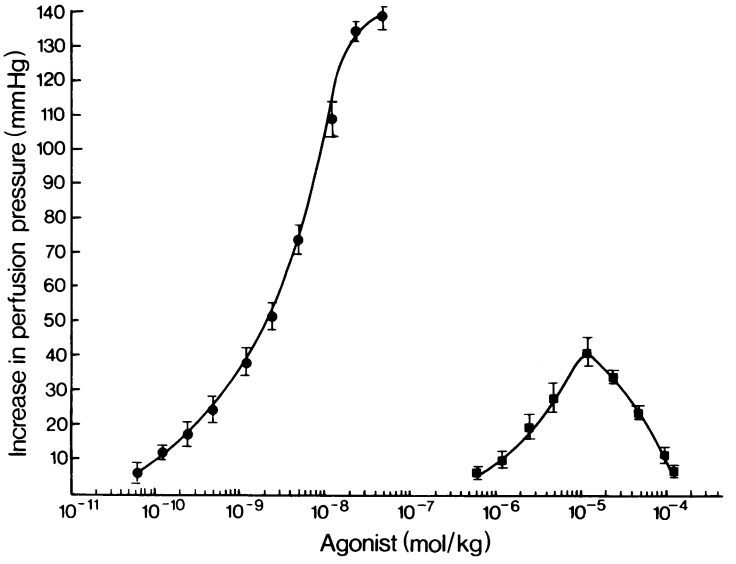


Figure 5 The constrictor activity of noradrenaline (●) and Wy 20051 (■) on the perfused mesenteric vasculature of the rat. The preparation was set up as described in the text. In contrast to noradrenaline, high doses of Wy 20051 produced a diminished constrictor response. At the dose level producing a 30 mmHg pressor response, Wy 20051 ($n=8$) was 0.82×10^4 times less potent than noradrenaline ($n=8$).

The maximum response to Wy 20051 (32.8 ± 5.0 arbitrary units; $n=5$) was significantly less than that produced by noradrenaline (65.0 ± 3.1 arbitrary units; $n=5$) ($P<0.001$).

The rat perfused mesenteric vasculature

Constrictor action of Wy 20051. Noradrenaline (5.9×10^{-11}–4.7×10^{-8} mol) and Wy 20051 (5.9×10^{-7}–1.2×10^{-4} mol) evoked constrictor responses of the perfused mesentery (Figure 5). The amplitude of the responses to Wy 20051 reached a maximum at 1.2×10^{-5} mol, but the maximum response (41.0 ± 4.3 mmHg; $n=6$) was significantly less than that produced by noradrenaline (137.0 ± 2.5 mmHg; $n=6$) ($P<0.001$). At higher doses the responses to Wy 20051 were reduced. The constrictor effect was absent at doses of 1.2×10^{-4} mol Wy 20051.

Phenoxybenzamine. In preliminary experiments phenoxybenzamine abolished noradrenaline-induced constrictor responses at a concentration of 1.0×10^{-7} mol/litre. Phenoxybenzamine (1.0×10^{-5} mol/litre) abolished responses to noradrenaline and 5-HT, but KCl, $BaCl_2$ and Wy 20051-induced constrictor responses were unchanged.

5-Hydroxytryptamine. Tachyphylaxis of mesenteric 5-HT receptors was produced by perfusing the tissue with 5-HT (2.8×10^{-5} mol/litre) in Krebs solution. An initial constriction occurred that gradually declined as the tachyphylaxis developed. When the perfusion pressure had returned to its basal value (approx. 1 h) the agonist sequence was repeated. The responses to noradrenaline, KCl, $BaCl_2$ and Wy 20051 were unchanged.

Calcium-free Krebs solution. Perfusion of the mesentery with calcium-free Krebs solution resulted in diminution and finally abolition of responses induced by noradrenaline, 5-HT, KCl and Wy 20051. Responses to $BaCl_2$ were unaffected. The mean ($n=5$)

Table 1 Abolition times for mesenteric pressor responses to submaximal doses of noradrenaline, KCl and Wy 20051 in the presence of calcium-free Krebs solution

Agonist	*Abolition time (min ± s.e. mean)*
KCl	47.4 ± 11.2
Wy 20051	97.8 ± 6.7
Noradrenaline	123.4 ± 9.2

Significance: KCl < Wy 20051 ($P<0.01$); KCl < noradrenaline ($P<0.001$); Wy 20051 *v.* noradrenaline NS.

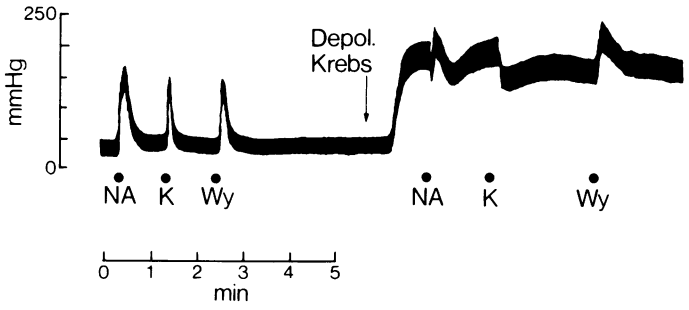


Figure 6 The effect of a depolarizing (high K^+) solution (see text) on mesenteric constrictor responses to submaximal doses of noradrenaline (NA, 2.4×10^{-9} mol), KCl (K, 1.1×10^{-5} mol) and Wy 20051 (Wy, 5.9×10^{-6} mol). Constant, approximately equieffective doses to the three agonists were obtained. The depolarizing solution induced a sustained contracture of the preparation. Under these conditions responses to KCl were abolished, but noradrenaline and Wy 20051 were still effective, although the responses were reduced.

times taken for total abolition of responses to noradrenaline, KCl and Wy 20051 are summarized in Table 1.

Depolarizing solution. A sustained contraction of the mesenteric vasculature was induced by the depolarizing solution described in the methods section ($n = 4$). Responses to doses of both noradrenaline and Wy 20051, which before depolarization had been equieffective, could be superimposed upon this contraction and were equally reduced in magnitude. In contrast no response was obtained to KCl (Figure 6).

Effect of γ-aminobutyric acid. The perfusion pressure of the isolated mesentery was unaltered by GABA (1.9×10^{-7}–1.9×10^{-4} mol).

α-Adrenoceptor antagonism. High doses of Wy 20051 (4.7×10^{-5} mol) did not reduce the response of the mesenteric vasculature to subsequent doses of noradrenaline (4.7×10^{-9} mol) or KCl (1.1×10^{-5} mol). However, a single dose of phenoxybenzamine (3.3×10^{-10} mol) abolished the responses to noradrenaline leaving those to KCl unchanged.

Discussion

Wy 20051 elicited pressor responses in the intact anaesthetized ganglion blocked rat, constrictor responses in the isolated perfused mesenteric vasculature preparation of the rat and evoked contractions of the guinea-pig isolated aortic strip. The possibility that Wy 20051 was indirectly stimulating α-receptors by causing release of noradrenaline at sympathetic nerve endings was discounted since Wy 20051-induced pressor responses were potentiated in reserpine-treated rats compared with normal control animals. In contrast tyramine-induced responses were abolished except at high doses where the effect could be ascribed to a direct action on the vascular smooth muscle (Krzanowski & Woodbury, 1966).

The reason for the potentiation of responses to Wy 20051 is not certain, but a non-specific supersensitivity to vasoactive agents other than catecholamines can result from reserpine treatment (Hudgins & Fleming, 1966). It is well established that catecholamine depletion is accompanied by a specific tissue supersensitivity to catecholamines (Burn & Rand, 1958; Shore, 1962) which may be caused, in the case of reserpine, by reduced overall catecholamine uptake, leaving more free catecholamine to react with tissue receptors. Trendelenburg (1966), whilst considering denervation supersensitivity in the nictitating membrane preparation, distinguished two components of supersensitivity, presynaptic and postsynaptic. Presynaptic supersensitivity is probably responsible for the specific effect on catecholamines whilst the non-specific supersensitivity for agents not taken up by adrenergic nerves such as peptides and, presumably, Wy 20051 may be due to vascular postsynaptic denervation supersensitivity. (Somlyo, Vinall & Somlyo, 1969.) Recent experiments show that reserpine increases the permeability of the muscle to calcium and acts, in addition, at some other calcium site to increase the availability of calcium for contraction (Carrier & Jurevics, 1973).

Direct stimulation of the α-adrenoceptor by Wy 20051 was considered to be unlikely since mesenteric constrictor responses to Wy 20051 could still be obtained in the presence of high concentrations of phenoxybenzamine (1.0×10^{-5} mol/litre); in contrast noradrenaline-induced responses were completely abolished by (1.0×10^{-7} mol/litre) phenoxybenzamine. Bevan, Osher & Su (1963) reported that high con-

centrations of phenoxybenzamine depressed responses of the rabbit aorta to K^+ probably by interference with transmembrane Ca^{2+} fluxes. This effect was not observed in the experiments on the mesentery although blockade of 5-HT-induced responses occurred (Nickerson, 1970).

Tachyphylaxis of the mesenteric 5-HT receptors to subsequent 5-HT-induced responses left Wy 20051-induced responses unchanged. It is therefore most unlikely that either α-adrenoceptor or 5-HT receptor stimulation is involved in the pressor action of this compound.

Perfusion of the mesenteric vasculature with calcium-free Krebs solution blocked noradrenaline, 5-HT, KCl and Wy 20051-induced responses, but did not inhibit $BaCl_2$-induced responses. $BaCl_2$-induced contractions seem to relate causally to inward movements of Ba^{2+}, which serve to function as a substrate for Ca^{2+} in the activation of contractile proteins (Toda, 1973). However, Ba^{2+} may also cause the release of Ca^{2+} (Bohr, 1964). The constrictor action of Wy 20051 unlike that of $BaCl_2$ seems to be calcium-dependent.

There is evidence that drugs can affect excitation contraction coupling by a common mechanism that is not entirely dependent on depolarization of the membrane. Thus noradrenaline responses on the rat tail artery persist long after the loss of the contractile response to high $[K^+]$ in a calcium-free medium (Hinke, 1965). In this series of experiments the time course for the abolition of Wy 20051-induced responses was significantly longer than that for KCl, but not significantly different from that of noradrenaline (Table 1). Furthermore, in the depolarized preparation, doses of noradrenaline and Wy 20051 that had evoked equal responses before depolarization continued to give equal, but reduced responses. However, responses could not be elicited for KCl (Figure 6). This phenomenon has been observed with maximal responses to different drugs in vascular smooth muscle (Somlyo & Somlyo, 1968a). These characteristics of pharmacomechanical coupling have been extensively reviewed by Somlyo & Somlyo (1968b, 1970) and have been ascribed to a longer and more persistent increase in the permeability of the membrane to calcium than is produced by depolarization, rather than an ability of the drugs to translocate Ca^{2+} into the cytoplasm from a compartment not accessible to depolarization. They proposed that membrane bound Ca^{2+} regulates the permeability of the membrane to ionized calcium itself. Depolarization removes some membrane bound calcium which causes increased permeability. Drugs acting by a pharmacomechanical mechanism release membrane bound calcium or eliminate its stabilizing effect with or without removing it from bound sites. The fact that noradrenaline produced consistently greater maximal responses than Wy 20051 both *in vivo* and *in vitro* suggests that noradrenaline was more able to overcome the stabilizing action of calcium and thereby produce a sustained increase in membrane permeability.

The tachyphylaxis of Wy 20051 pressor responses induced by high doses of the compound (3.8×10^{-5}–3.0×10^{-4} mol/kg) in intact animals is interesting since noradrenaline responses are also significantly reduced when preceded by doses of this order (3.4×10^{-5} mol; Figure 2). However, no demonstrable α-adrenoceptor antagonism could be detected in the isolated perfused mesenteric vasculature with even larger bolus doses of Wy 20051 known to produce tachyphylaxis in this preparation (4.7×10^{-5} mol; Figure 5). In contrast, a single bolus dose of phenoxybenzamine (3.3×10^{-10} mol) abolished the mesenteric pressor response induced by noradrenaline (4.7×10^{-9} mol). These experiments suggest that the reduction of Wy 20051 and noradrenaline responses *in vivo* occurring when these agonists are preceded by supramaximal doses of Wy 20051 do not directly involve the α-adrenoceptor. Possible explanations for this tachyphylaxis include exhaustion of bound calcium by Wy 20051, or permanent damage of the cell membrane with consequent disruption of calcium fluxes.

The chemical resemblance of Wy 20051 to GABA does not seem to be important in the cardiovascular activity of this compound since GABA had no effect on the rat mesentery and reports suggest that GABA has a depressor effect on blood pressure which is dependent on an optimal distance between the amino acid and carboxyl groups (Takahaski, Tiba, Iino & Takayasu, 1955). The depressor action of GABA in the rabbit has been ascribed to an action on the medulla (Takahaski, Tiba, Yamazaki & Noguchi, 1958) and in dogs to peripheral ganglion blockade (Stanton & Woodhouse, 1959; Stanton, 1963). However, pressor effects have been observed in the dog and may be due to carotid and aortic chemoreceptor stimulation.

In conclusion, Wy 20051 showed novel pharmacological activity in causing pressor responses in the intact animal and constrictor responses in the isolated perfused mesenteric vascular bed and guinea-pig isolated aorta. The contractions are calcium-dependent and are due, at least in part, to an action on pharmacomechanical coupling in the vascular smooth muscle.

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