Drug-induced rhythmicity in smooth muscle

SIR,—When isolated in organ-bath experiments, the vas deferens of the guinea-pig or rat is usually quiescent, but several workers have reported the initiation of rhythmical contractions by drugs (Birmingham & Wilson, 1963; Boyd, Burnstock & others, 1963; Ohlin & Strömblad, 1963; Burnstock & Holman, 1964; Bentley, 1965, 1966). In the experiments described here, cocaine, procaine and lignocaine induced reproducible contractile activity.

Adult guinea-pigs (400–750 g) and rats (200–300 g) were killed by stunning and bleeding, and both vasa deferentia were removed. These were suspended in Krebs solution at 32°, oxygenated with 95% oxygen and 5% carbon dioxide. Recording of contractions was with frontal writing levers on smoked paper, using a 600 mg load and 5 × magnification.

Test vasa were exposed to increasing concentrations of drug, and it was noted that activity was initiated at a threshold concentration (Table 1). This activity consisted of rhythmical contractions and relaxations (Fig. 1), and with each successive increase in concentration, an increase in amplitude or frequency,

TABLE 1. INITIATION OF RHYTHMICAL ACTIVITY. ACTIVITY THRESHOLD AND OPTIMUM CONCENTRATIONS (MOLAR) OF COCAINE, PROCAINE AND LIGNOCAINE

	Guinea-pig				Rat			
	Normal		Denervated		Normal		Denervated	
	Threshold	Optimum	Threshold	Optimum	Threshold	Optimum	Threshold	Optimum
Cocaine	1×10^{-4}	1×10^{-3}	5×10^{-4}	1×10^{-3}	1×10^{-5}	1×10^{-3}	1×10^{-4}	1×10^{-3}
Procaine	1×10^{-3}	1×10^{-2}	1×10^{-3}	1×10^{-2}	5×10^{-4}	5×10^{-3}	1×10^{-4}	1×10^{-3}
Lignocaine	2×10^{-4}	5×10^{-3}	1×10^{-3}	5×10^{-3}	1×10^{-4}	1×10^{-3}	5×10^{-5}	1×10^{-3}

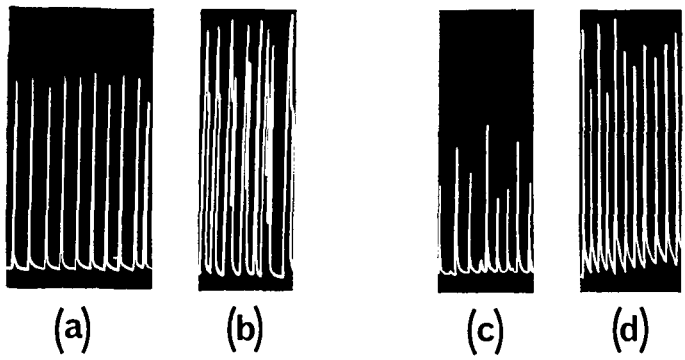

FIG. 1. Response of the guinea-pig vas deferens to local anaesthetics. (a) Rhythmical contractions produced by the guinea-pig vas deferens immersed in 5×10^{-3}M lignocaine. Such activity was often seen to persist for many hours. (b) Rhythmical contractions produced in 2×10^{-3}M procaine. The multiphasic contractions were often seen when high concentrations of cocaine, procaine and lignocaine were used. (c) Activity produced in response to 1×10^{-3}M lignocaine. This specimen had intact intramural nerves at the time of removal. (d) The denervated partner of the specimen in (c) produced considerably more activity in 1×10^{-3}M lignocaine. The denervation was of 8 days' duration.

or both, was noted. The notable feature of the responses obtained after the application of drug was their amplitude and frequency, and their similarity to those obtained from the electrically stimulated vas, (Fig. 1a). An optimum drug concentration was usually obtained, after which the activity rapidly declined. Activity could be terminated at any point by washing out the drug with normal Krebs solution, and could be immediately restituted by the return of the drug. It may therefore be assumed that the contractions were initiated by the drug.

A control vas, not exposed to drug, remained inactive throughout the duration of each experiment. Generally speaking, the vas deferens of the rat appeared to be the more sensitive for the threshold and optimum drug concentrations, although the actual contractions were smaller than those of the guinea-pig.

On the basis of the amount of activity, the most potent agent tried appeared to be procaine (Fig. 1b), but several other drugs also produced a great deal of activity. These were piperoxan, thymoxamine and mepyramine. The ability to produce rhythmical activity, and the local anaesthetic properties do not seem to be related, since some agents which produced potent local anaesthesia were unable to stimulate the tissue. For example, benzocaine, chlorcyclizine and chlorpromazine were ineffective.

Is the phenomenon dependant on the nerve-supply to the tissue? That this does not seem to be so is indicated by experiments made on surgically denervated tissue.

Denervated vasa were obtained from guinea-pigs and rats which had one vas denervated eight days previously (Birmingham, 1967, 1968). When the denervated vas was set up alongside its innervated partner from the same animal, both became rhythmically active when exposed to drug. The denervated vas was usually more active at its threshold and optimum drug concentrations than its control. These results indicate that an intact intramural nervous system in the tissue is not essential for the initiation of the type of activity described.

In addition, vasa from animals pretreated with reserpine were still able to achieve as much activity as their controls. That catecholamines are not necessary for this activity was indicated by histochemical studies using a modified Falck's fluorescence technique (Spriggs, Lever & others, 1966), which showed complete depletion of fluorescent nerve-fibres in the tissue after reserpine treatment.

It seems legitimate to conclude that drugs like procaine, cocaine and lignocaine which activate the tissue in the manner described, do so by an action on the smooth muscle cells which is independent of their innervation.

Acknowledgement. The author wishes to thank Dr. A. T. Birmingham for supplying the denervated and reserpinized animal specimens, and the Medical Research Council for the Scholarship which enabled this work to be carried out.

Department of Pharmacology,
King's College,
Strand, W.C.2, England.
April 30, 1968

G. S. CLIFF

References

Bentley, G. A. (1965). *Br. J. Pharmac. Chemother.*, **25**, 243–256.
Bentley, G. A. (1966). *Ibid.*, **27**, 64–80.
Birmingham, A. T. (1967). *J. Physiol., Lond.*, **190**, 16P–17P.
Birmingham, A. T. (1968). *Ibid.*, **197**, 37P–38P.

Birmingham, A. T. & Wilson, A. B. (1963). *Br. J. Pharmac. Chemother.*, **21**, 569–580.
Boyd, H., Burnstock, G., Campbell, G., Jowett, A., O'Shea, J. & Wood, M. (1963). *Ibid.*, **20**, 418–435.
Burnstock, G. & Holman, M. E. (1964). *Ibid.*, **23**, 600–612.
Ohlin, P. & Strömblad, B. C. R. (1963). *Ibid.*, **20**, 299–306.
Spriggs, T. L. B., Lever, J. D., Rees, P. M. & Graham, J. D. P. (1966). *Stain Tech.*, **41**, 323–327.

Enhanced toxicity of imipramine and desipramine in aggregated mice

SIR,—From experiments with amphetamine (Cohen & Lal, 1964) and cocaine (Lal & Chessick, 1965), it was postulated that the enhanced toxicity of these drugs in aggregated mice was related to their common property by which they inhibit tissue uptake of catecholamines. Recently, imipramine and desipramine were found to block tissue uptake of noradrenaline *in vitro* (Iversen, 1965) and *in vivo* (Glowinski & Axelrod, 1964). The present work shows that aggregation of mice enhanced the toxicity of both drugs.

Swiss albino random-bred male mice of 22–28 g were placed in stainless steel cages (7 × 9·5 × 7 inches), 10 to a cage, 2 hr or more before the intraperitoneal administration of the drugs. Immediately after injection the animals were returned to the same cages, one mouse to a cage for isolation and 10 mice to a cage for aggregation. To maintain group size during the experiment, any dead mouse was replaced by another living animal.

Data summarized in Fig. 1 show that aggregation enhanced the acute lethality of imipramine and desipramine. Imipramine was less toxic than desipramine. Table 1 shows that the onset of clonic convulsions or death after a large dose of desipramine was significantly sooner after aggregation.

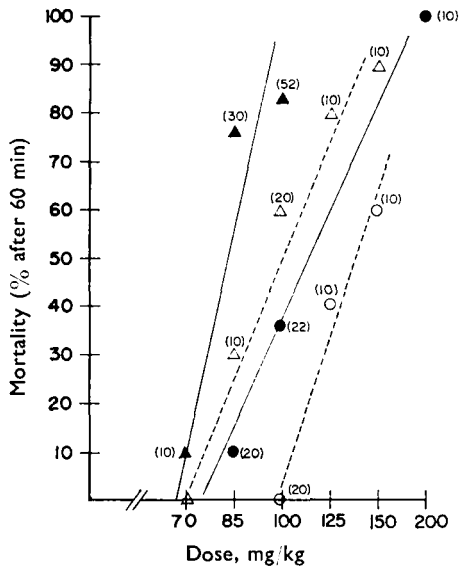

FIG. 1. Toxicity of imipramine and desipramine (doses as mg/kg) in aggregated and isolated mice. —▲— Desipramine aggregated. —●— Desipramine isolated. - - -△- - - Imipramine aggregated. - - -○- - - Imipramine isolated.