Lowering of rat brain 3-methoxy-4-hydroxyphenylethylene glycol sulphate (MOPEG sulphate) concentration by 2,6-dichlorobenzylidene aminoguanidine

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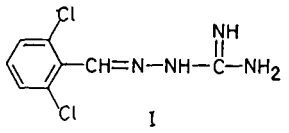
Baum, Shropshire & others (1970) have shown that 2,6-dichlorobenzylidene aminoguanidine (I) lowers blood pressure in experimental animals, and Bolme, Corrodi & Fuxe (1973) presented evidence that the mechanism might involve stimulation of central noradrenergic receptors. Generally, stimulants of neurotransmitter receptors in brain decrease the turnover and release of the neurotransmitter. Bolme & others (1973) showed that 2,6-dichlorobenzylidene aminoguanidine slowed the decline in noradrenaline after its synthesis was inhibited, suggesting noradrenaline turnover was decreased. Steady state concentration of 3-methoxy-4-hydroxyphenylethylene glycol sulphate (MOPEG sulphate) has been suggested to be an index of noradrenaline turnover in rat brain (Meek & Neff, 1973).



I

Clonidine, a drug whose hypotensive effect is thought to be mediated by stimulation of central noradrenergic receptors (see Scriabine, Clineschmidt & Sweet, 1976), has been shown to lower MOPEG sulphate concentration in rat brain (Braestrup, 1974; Braestrup & Nielsen, 1976). For these reasons, we determined the effect of 2,6-dichlorobenzylidene aminoguanidine on MOPEG sulphate (and noradrenaline) concentration in rat brain.

Male Wistar rats, 130–150 g (Harlan Industries, Cumberland, Indiana) were kept in hanging wire cages in groups of 5 with free access to food and water. 2,6-Dichlorobenzylidene aminoguanidine hydrochloride was injected in acacia suspension (injection volume 1 ml kg^{-1}, i.p.). Rats were decapitated, and whole brains quickly excised and frozen on dry ice. Spectrofluorometric methods were used to estimate noradrenaline (Chang, 1974) and MOPEG sulphate (Meek & Neff, 1972).

Table 1 shows that 2,6-dichlorobenzylidene aminoguanidine lowers MOPEG sulphate concentration in rat brain while causing little or no change in noradrenaline concentration. In the first experiment, a high dose maximally depressed MOPEG sulphate at 4–8 h. In the second experiment, doses of 1·25–20 mg kg^{-1} produced similar degrees of depression in MOPEG sulphate. Noradrenaline concentration was changed minimally or not at all in these experiments and was not measured subsequently. The third experiment shows a dose-related lowering of MOPEG sulphate (statistically significant only at the 1 mg kg^{-1} dose), and the fourth experiment shows a duration of effect at that dose similar to the duration of a higher dose.

Table 1. *Effect of 2,6-dichlorobenzylidene aminoguanidine on MOPEG sulphate and noradrenaline concentrations in rat brain: dose-dependence and duration of effects.*

Exp. No.	Time (h)	Dose, (mg kg^{-1})	MOPEG SO_4 (ng g^{-1})		Noradrenaline (μg g^{-1})	
1.	0	—	118±3		0·47±0·01	
	1	20	95±6	(−18%)	0·50±0·01	
	2	20	83±3	(−29%)	0·54±0·02	(+15%)
	4	20	63±3	(−46%)	0·51±0·02	
	8	20	54±3	(−54%)	0·50±0·02	
	16	20	106±13		0·44±0·01	
	24	20	117±4		0·43±0·01	
2.	4	0	151±4		0·45±0·01	
	4	1·25	81±5	(−46%)	0·46±0·02	
	4	2·5	89±7	(−41%)	0·43±0·01	
	4	5	77±8	(−49%)	0·44±0·01	
	4	10	79±5	(−48%)	0·46±0·01	
	4	20	70±5	(−54%)	0·45±0·01	
3.	4	0	131±11			
	4	0·1	124±5			
	4	0·3	112±5			
	4	1	101±5	(−23%)		
4.	0	—	118±3			
	1·5	1	116±5			
	2·75	1	85±2	(−28%)		
	5	1	85±8	(−28%)		
	6·5	1	71±9	(−40%)		
	8	1	87±9	(−26%)		

Mean values ± standard errors for 5 or 10 rats per group are shown. Numbers in parenthesis show percentage changes in all cases in which a drug effect was statistically significant ($P<0·05$).

Our results support the claim of Bolme & others (1973) that 2,6-dichlorobenzylidene aminoguanidine reduces noradrenaline turnover in rat brain, perhaps by stimulating noradrenergic receptors and thereby eliciting a feedback mechanism that suppresses noradrenaline synthesis and release. The duration of lowering of MOPEG sulphate by 2,6-dichlorobenzylidene aminoguanidine was similar to that produced by clonidine as reported by Braestrup & Nielsen (1976) and confirmed by us (unpublished data).

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REFERENCES

BAUM, T., SHROPSHIRE, A. T., ROWLES, G., VAN PELT, R., FERNANDEZ, S. P., ECKFELD, D. K. & GLUCKMAN, M. I. (1970). *J. Pharmac. exp. Ther.*, **171**, 276–287.

BOLME, P., CORRODI, H. & FUXE, K. (1973). *Eur. J. Pharmac.*, **23**, 175–182.

BRAESTRUP, C. (1974). *J. Pharm. Pharmac.*, **26**, 139–141.

BRAESTRUP, C. & NIELSEN, M. (1976). *J. Pharmac. exp. Ther.*, **198**, 596–608.

CHANG, C. C. (1964). *Int. J. Neuropharmac.*, **3**, 643–649.

MEEK, J. L. & NEFF, N. H. (1972). *Br. J. Pharmac.*, **45**, 435–441.

MEEK, J. L. & NEFF, N. H. (1973). *J. Pharmac. exp. Ther.*, **184**, 570–575.

SCRIABINE, A., CLINESCHMIDT, B. V. & SWEET, C. S. (1976). *A. Rev. Pharmac. Toxic.*, **16**, 113–123.

Effects of sympathetic nerve stimulation on electrical activity of Auerbach's plexus and intestinal smooth muscle tone

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It is well known that movement of an intestine is inhibited by sympathetic nerve stimulation. The site of action of catecholamines released by sympathetic nerve stimulation is, however, obscure. Recent histochemical studies indicated that fluorecence of catecholamines was observed mainly around nerve cells in Auerbach's plexus (Norberg, 1964; Jacobowitz, 1965). These observations suggested that the site of action of catecholamines released from the sympathetic nerve endings might be in Auerbach's plexus (Norberg & Sjoqvist, 1966). The present experiments were designed to test effects of sympathetic nerve stimulation on electrical activity of Auerbach's plexus and tone of intestinal smooth muscle.

Male guinea-pigs, 350 to 600 g, were killed by a blow on the head; from a small piece of ileum, longitudinal muscle with perivascular nerve was dissected (Finkleman, 1930) and mounted in a rectangular Lucite chamber filled with 10 ml of Locke Ringer solution at $36 \pm 0{\cdot}5°$ and gassed with 5% CO_2 in oxygen. Both ends of the preparation were sewn with stainless pins to spread the preparation. One end was fixed to the chamber and the other was attached to an isometric force-displacement transducer (Nihon Koden, SS-IT) connected to a recorder. Resting tension was adjusted to 1 g. Electrical activity of Auerbach's plexus was simultaneously recorded by a floating fine glass suction electrode (Tip diameter: 30 to 100 μm) according to Sato, Takayanagi & Takagi (1973). To stimulate the sympathetic nerve, square-wave monophasic pulses of 0·3 to 1 ms duration at 40 Hz were applied to the perivascular nerve for 0·5 to 10 s at supra-maximal voltage through Ag-AgCl electrodes. Those preparations that were relaxed by sympathetic nerve stimulation were used. Locke Ringer solution used had the following composition (mM): NaCl 154, KCl 5·6, $CaCl_2$ 2·2, MgCl 2·1, $NaHCO_3$ 5·9 and glucose 2·8, the pH was 7·8.

Drugs used were guanethidine sulphate (Nippon Ciba Geigy Co. Ltd, Japan), nicotine bitartrate (Nakarai

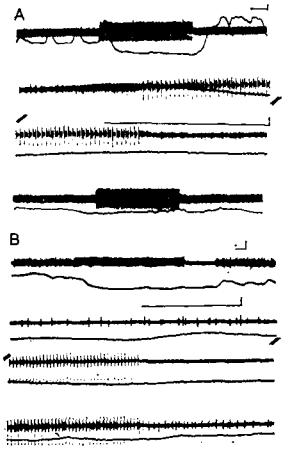

FIG. 1. Effects of sympathetic nerve stimulation on electrical activity of Auerbach's plexus and tension of longitudinal muscle. Upper trace: electrical activity. Lower trace: tension. Vertical calibration: 2 μV and 100 mg. Horizontal calibration: 1 s. Large spikes are artifacts originated in electrical stimulation.
A: Type 1 responses. The top pair: control responses to sympathetic nerve stimulation. The second and third pairs: control responses recorded at a high sweep velocity to observe the spike between stimuli. Note that spikes from Auerbach's plexus were unaffected, notwithstanding that intestinal tone was decreased under and after sympathetic nerve stimulation. The bottom pair: responses in the presence of guanethidine (3×10^{-6} g ml^{-1}).
B: Type 2 responses. The top pair: control responses to sympathetic nerve stimulation. The second and third pairs: control responses recorded at a high sweep velocity. Note that the spike frequency was greatly reduced under and after sympathetic nerve stimulation. The bottom pair: responses recorded at a high sweep velocity in the presence of guanethidine (3×10^{-6} g ml^{-1}).

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