Short communications

Supersensitivity to the negative chronotopic action of carbachol and methacholine in the rat

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Pretreatment of rats for 6 days with the ganglion blocking agent, chlorisondamine, produces a 2–3 fold increase in the sensitivity of the heart to the negative chronotropic action of carbachol and methacholine.

If a tissue is disconnected from the central nervous system by surgical means or by the use of drugs which interfere with neural transmission, nerve impulses do not reach the tissue and the tissue becomes supersensitive to chemical stimuli (Cannon & Rosenblueth, 1949; Emmelin, 1961; Trendelenburg, 1963). A reduction in the activity of the tissue, caused by interruption of transmission, is thought to be a critical factor in the development of supersensitivity (Trendelenburg & Weiner, 1962; Reas & Trendelenburg, 1967; Fleming, 1968). In virtually all of the studies of this phenomenon, however, the drugs used to detect supersensitivity have been those which increase the activity of a tissue (for example contraction of muscle, acceleration of heart rate, enhancement of glandular secretion). Little attention, however, has been paid to the inhibitory action of drugs and it would be of interest to know whether supersensitivity develops to the inhibitory action of drugs as well as to the excitatory action. The only report on this aspect of supersensitivity is from Fleming & Schmidt (1962) who found supersensitivity to the inhibitory action of sympathomimetics on the rabbit ileum after subacute administration of reserpine.

The present study represents an attempt to detect supersensitivity to the inhibitory effects of cholinergic agents on the rat heart after administration of a ganglionic blocking agent for 6 days.

Methods.—Female Wistar rats weighing 200–250 g were treated twice daily for 6 days with 10 mg/kg of chlorisondamine chloride. Preliminary experiments revealed that 5 mg/kg of chlorisondamine produced maximal dilation of the pupil for 3–5 h indicating effective ganglion blockade. A dose of 10 mg/kg was chosen in order to ensure ganglion blockade in all of the animals. The drug was given subcutaneously and the injections were spaced 10–12 h apart. Control rats received equivalent volumes of 0·9% NaCl. Approximately 12 h after the last injection, the rats were anaesthetized with urethane, 1·5 g/kg, which was given intraperitoneally. Five minutes later the rats received a final injection of chlorisondamine. In addition, control rats, which up to this point had received no drug, were also given chlorisondamine, 10 mg/kg. The rats were then prepared for recording blood pressure and electrocardiogram by methods which have already been described (McPhillips & Dar, 1967). Heart rate was determined from the electrocardiogram. Carbachol chloride and methacholine chloride were given intravenously through a polyethylene catheter inserted into the external jugular vein. The volume of injection was 0·15 ml or less. The catheter was immediately flushed with an equivalent volume of 0·9% NaCl. Dose-response curves were constructed on semilogarithmic paper by plotting decreases in heart rate, in beats/min, against the dose of carbachol or methacholine. Comparisons between control groups and those pretreated with chlorisondamine were made on the basis of the ED 125 value. The ED 125 is the effective dose, in μmol/kg, required to reduce the heart rate by 125 beats/minute. Since the resting heart rates of the rats, under anaesthesia, were approximately 250 beats/min, the ED 125 value represents the midpoint of the dose-response curve. The ED 125 was considered to be a valid basis for comparison since dose-response curves obtained from treated and control groups were parallel.

Experiments were also done with isolated spontaneously beating rat atria. The rats were treated in a manner identical to that described above, and were killed by cervical dislocation; the right atrium was removed and set up in an organ bath. Sensitivity to carbachol was measured by the method described by Perrine & McPhillips (1970). Comparison between control and treated groups was made on the basis of the ED 125 value.

All mean ED 125 values reported in this paper are geometric means with 95% confidence intervals.

Results.—Subacute administration of the ganglion-blocking drug, chlorisondamine, did not significantly affect the resting heart rate of the rats (Table 1).

A comparison of the ED125 values shows that the subacute administration of chlorisondamine produces supersensitivity to carbachol and methacholine. The rats treated subacutely with chlorisondamine were 3 times as sensitive to the negative chronotropic action of carbachol and twice as sensitive to the negative chronotropic action of methacholine as control rats (Table 1). The results indicate, therefore, that supersensitivity does develop to the negative chronotropic action of cholinergic agents.

Supersensitivity could not, however, be detected in the isolated spontaneously beating atrium. There was no significant effect of chlorisondamine pretreatment on the spontaneous rate of beating (Table 1); nor was the ED 125 for carbachol affected by the pretreatment.

Discussion.—This study has shown that supersensitivity can develop to the inhibitory action of cholinergic drugs. Pretreatment for 6 days with the ganglion blocking agent, chlorisondamine, produced supersensitivity to the negative chronotropic effect of carbachol and methacholine in the rat. The sensitivity of the heart to these drugs increased 2–3 fold.

It might be argued that the effect observed was not true supersensitivity. Ganglion blockade inhibits cardiovascular reflexes. Thus, reflex tachycardia, which is triggered by a fall in blood pressure, is inhibited by ganglionic blocking agents, and the direct negative chronotropic action of cholinergic drugs together with inhibition of reflex tachycardia, would cause an animal treated with a ganglion blocking drug to appear supersensitive to negative chronotropic agents. In the present study, however, the control rats were also dosed with 10 mg/kg of chlorisondamine given immediately before the experiment. If simple ganglion blockade were responsible for supersensitivity there should have been no difference between treated and control groups.

Supersensitivity could not be detected *in vitro*, but there have been other instances in which a similar discrepancy has been detected between results obtained *in vivo* and *in vitro*. For example, Tsai, Denham & McGrath (1968) were able to detect supersensitivity of the nictitating membrane in the intact animal but not in the smooth muscle of the membrane after it had been isolated from the animal. Westfall & Fleming (1968) studied supersensitivity to catecholamine in the guinea-pig, and demonstrated supersensitivity to noradrenaline in the spinal animal and in the isolated perfused heart but not in the isolated atrium. They suggested that the occurrence of supersensitivity was inversely related to the amount of trauma and manipulation to which a tissue was subjected during isolation. The ion content of a tissue is known to change when it is isolated and Dawkins & Bohr (1960) have reported that altered cation composition appears to be related to the

TABLE 1. *Effect of subacute administration of chlorisondamine on the sensitivity of the rat heart to carbachol and methacholine*

Preparation	Drug	Number of rats	Resting rate (beats/min) ±S.E.M.	ED 125 (μmol/kg)	95% confidence limits
Anaesthetized rat	Carbachol				
	Control	6	298±12	17	11–27
	Chlorisondamine treated	8	270±14	6	4–9*
Anaesthetized rat	Methacholine				
	Control	7	282± 7	30	21–44
	Chlorisondamine treated	8	278±17	14	10–20*
Isolated atria	Carbachol			μmol/l.	
	Control	8	294± 8	0·7	0·5–0·9
	Chlorisondamine treated	5	275± 9	0·6	0·4–0·9

* Significantly different from control value at the 1% level of probability.

mechanical handling of a tissue rather than to the different chemical environment.

The results of the present study, as well as the report by Fleming & Schmidt (1962), demonstrate that supersensitivity can develop to the inhibitory, as well as to the excitatory, action of drugs.

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