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Reversible Nature of Atropine Blockade of Acetylcholine in Vascular Beds* (33361)

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During the course of some studies of autonomic nervous system function, we used atropine to prevent the cardiovascular effects of injected acetylcholine. Atropine never failed to produce the familiar persistent blockade of cholinergic effects on the heart, but the blockade of cholinergic effects on blood vessels appeared to be temporary. That is, the first postatropine injection of acetylcholine produced no vasodilation, while a second or third usually did. This was unexpected because while the very substantial literature on the pharmacology of atropine contains many references to variation in sensitivity of cholinergic receptors to atropine, it does not include any reports of an atropine blockade which is reduced or terminated after several injections of acetylcholine. In this report we present data from a large number of experiments which document this effect.

Materials and Methods. Cats anesthetized with pentobarbital sodium (30 mg/kg) were used. Bood pressure was recorded from the femoral artery by means of a Statham pressure transducer connected to a Sanborn end-recorder. Vascular changes in local beds were monitored by the electrical impedance technique, the basic principles of which have

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been described in Nyboer's monograph (1). The special adaptations used in this study have been reported by Neidle and Liebman (2). The method involves the passage of a radio frequency current of 50–175 ks of about 4 mW through the tissue under investigation. Changes in the impedance or resistance to the flow of current can be recorded from suitably designed electrodes. Changes in impedance are reflected by changes in pulse amplitude, each pulse representing a balance between inflow and outflow of blood during a single cardiac cycle. An increase in the impedance pulse amplitide is interpreted to indicate increased volume of the part or increased volume flow, and a decrease in impedance pulse amplitude signifies decreased volume or flow. The vascular beds from which we recorded were: ear, spleen, and skin and muscle of various parts of the face and forelimb. Drugs were injected via a catheter placed in the saphenous vein. The drugs used were: acetylcholine (ACh), methacholine, pilocarpine, atropine, scopolamine, and homatropine. The right vagus nerve was exposed in the neck and stimulated peripherally with a Grass square wave stimulator to test for blockade of vagal transmission.

Results. In 22 cats intravenous injection of 5–20 μg/kg of ACh produced the typical fall in arterial pressure, vasodilatation in most monitored beds, and slight, transient cardiac slowing with the higher doses. Atropine (2 mg/kg) was administered intravenously and cholinergic blockade was confirmed by stimulation of the peripheral segment of the right vagus nerve. Administration of a dose of ACh equal to the control dose produced no perceptible cardiovascular changes. However, a second, third, or fourth dose of ACh, administered at 5 min intervals after atropinization, caused a fall in blood pressure and in most cases vasodilatation in one or more of the monitored beds. We will henceforth refer to this phenomenon as "breakthrough." The magnitude of the blood pressure fall (5–10 mm Hg) was never so great as that preceding atropinization (20–60 mm Hg). Subsequent injections of ACh failed to produce a significantly greater fall in blood pressure. There was no cardiac slowing in response either to ACh or vagal stimulation. The cardiovascular effects of acetylcholine, both before and after atropinization, were of short duration. Invariably, heart rate, blood pressure, and impedance pulse amplitude had returned to control levels within 30 sec.

In 9 cats an additional dose of atropine (1 mg/kg) was administered after breakthrough. In every case, the first dose of ACh after reatropinization produced no change in blood pressure; the second, third, or fourth dose, however, resulted in a fall in blood pressure and vasodilatation in some of the vascular beds. Atropine was administered up to 5 times in a single animal and in all cases breakthrough was observed.

We tested the role of time in this phenomenon. In 2 cats saline was injected 5 and 10 min postatropine, instead of ACh, and at 15 min the test dose of ACh was injected. No breakthrough occurred. Saline was given at 20 and 25 min, and at 30 min ACh was injected. In 1 of these breakthrough occurred after 30 min or upon the second injection of acetylcholine; in the other, breakthrough occurred at 45 min or after the third injection of ACh. In a third cat the first dose of ACh was given 15 min postatropine and had no effect. Breakthrough occurred 38 min later upon the third injection of ACh. In 2 other cats the interval between atropine and ACh was lengthened to 30 min. Breakthrough occurred at the third and fifth ACh injections or at 40 and 75 min respectively. Breakthrough after reatropinization occurred after the third ACh injection.

The atropine blockade was compromised just as effectively by small doses as by large doses of ACh. In most of our experiments, doses of 5–10 μg/kg were used and breakthrough occurred at the second, third, or fourth injections. However, in experiments using doses as high as 175 μg/kg it still took 2 or 3 doses to elicit breakthrough. Two typical sequences follow: injection of 20 and 50 μg/kg of ACh, breakthrough at the third injection of 100 μg/kg 20 min postatropine; injection of 100 μg/kg, breakthrough at the second injection of 150 μg/kg 13 min postatropine.

In order to determine if breakthrough is limited to the ACh–atropine relationship, we made substitutions for each in turn. In 4 experiments methacholine was used instead of ACh. In 9 trials with atropine, methacholine (1–20 μg/kg) breakthrough was observed only after the administration of massive doses of methacholine over a long period of time (8 cases) or never (1 case). In the 2 of these animals tested, on the other hand, minimal doses of ACh were capable of producing a fall in blood pressure and vasodilatation after 1 or 2 injections given at the customary 5 min intervals.

Pilocarpine (12.5–300 μg/kg) was substituted for ACh in 2 experiments, and the results were essentially the same as those obtained with methacholine, viz., long after atropinization and administration of many large doses of pilocarpine, atropine blockade persisted, even though in these same animals at the same time small doses of ACh quickly broke through the atropine blockade of vascular effects.

In the next series of 7 experiments scopolamine bromide (1 mg/kg) or homatropine bromide (1.6–2.0 mg/kg) was substituted for atropine. In 2 cats scopolamine prevented cardiac and vascular responses to ACh injection and vagal stimulation. In both cases the blockade persisted for the duration of the experiment, 27 and 41 min, respectively, despite the fact that increasingly large doses of ACh (up to 50 μg/kg) had been given at 5 min intervals. In 5 experiments homatropine never completely blocked the vasodilator response to injected ACh, even through cardiac blockade was well established.

Using an *in vitro* technique, we extended our observations on the stability of the ACh–atropine relationship to the smooth muscle of the rabbit duodenum. Duodenal segments (2.5–5 cm) from 3 rabbits were immersed in baths of aerated Hanks' solution, pH 7.2-7.4, and maintained at 35–38°. Recordings were made on smoked kymographs. The minimum dose of ACh which would produce changes in baseline tonus was determined for each muscle segment. It ranged from 0.005 to 0.02 μg/ml of bath. This dose was added to the bath 3 or 4 times, at approximately 5 min intervals, after which a single dose of atropine (0.01–0.2 μg/ml of bath) was added. When the muscle recovered its tonus, the fastness of the blockade was tested by repeated administration, at the usual 5 min intervals, of the minimum dose of ACh. In 106 postatropine administrations of ACh to 33 duodenal segments over a period ranging from 22 to 88 min, breakthrough never occurred. Even when the dose of ACh was increased by a factor of 2–5 times, the atropine blockade remained fast.

Discussion. The wide use of atropine as a pharmacologic tool is based on certain assumptions about its activity. Some of these are:

(i) Atropine is a cholinergic blocking agent which is more effective at postganglionic sites than at preganglionic or skeletal muscle sites.

(ii) Certain sites exhibit a degree of atropine resistance which is evidenced by the fact that atropine does not successfully block responses to nerve stimulation. Two examples of such atropine resistance are the failure of atropine to prevent motor responses of the bladder to direct cholinergic stimulation (3) and Heidenhain's report in 1872 that while atropine could prevent secretory responses to chorda tympani stimulation, it could not prevent vasodilatation. Atropine resistance has recently been evaluated by Ursillo (4).

(iii) ". . . the abolition of a nerve effect by atropine can be taken as strong evidence that the endings of that nerve are cholinergic. However, the converse is not always true." (5).

(iv) "Atropine in clinical doses completely counteracts the peripheral dilatation and sharp fall in blood pressure caused by choline esters." (6).

(v) The duration of action of atropine is *not* brief.

(vi) Atropine acts by a competitive blockade of the receptor site and the blockade can be overcome by increasing the concentration of ACh at receptor sites.

The data presented in this paper raise questions about some of these assumptions. For example, while it appears to be true that in most cases atropine effectively prevents

vasodilatation in response to a single small dose of ACh, subsequent doses are not blocked. This is clearly not the result of an impermanence in atropine's action, because at this same time atropine blockade at the heart and pupillary sphincter persists. Nor can it be said that the competitive blockade is being overwhelmed by accumulation of large amounts of ACh, for the doses of ACh used were minimal and the intervals between injections made it highly improbable that ACh could accumulate. The well-known rapidity of destruction of ACh by acetylcholinesterase and our experimental finding that the effects of ACh were never apparent for more than 30 sec make reasonable the assumption that ACh is not likely to be physiologically active for periods as long as 5-min after injection. In addition, the fact that a first injection of 100 μg/kg of ACh failed to break the atropine blockade, while a third or fourth injection of 5 μg/kg of ACh, for a total of 20 μg/kg, did break the blockade, makes any hypothesis based on ACh accumulation untenable. Nor does it seem likely that the vascular beds monitored in this study were exhibiting a form of atropine-resistance since initially they were effectively blocked by atropine. Finally, it cannot be argued that atropine blockade is affected by elapsed time, since blockade of the heart, pupil, and intestinal segments persisted for several hours and in vascular beds lasted for as long as 75 min in experiments in which only 1 or 2 doses of ACh had been administered during this period.

Our data suggest that atropine blockade in the vascular bed is broken neither by the passage of time, nor by the total dose of ACh, nor by the intervals between doses, but rather by the number of times ACh is allowed to compete for the receptor site. Breakthrough is not observed in other cholinergic sites, such as the smooth muscle of the intestine, the pupil, and the heart. This suggests the existence of 2 kinds of cholinergic receptors: those which are present in the above-mentioned organs and which appear to be atropine-fast and others, present in the vascular beds, which are engaged reversibly by atropine. Our data indicate that this reversible relationship does not extend to other chemically related agonist–antagonist systems, such as pilocarpine–atropine or ACh–scopolamine, etc. The facts that blood pressure fall in response to ACh after breakthrough is much smaller than before atropinization and that repeated administration of ACh after breakthrough does not increase the magnitude of blood pressure fall tempt us to postulate either that there are both kinds of receptors in vascular beds or that some vascular beds have atropine-fast receptors and others do not. The pattern of distribution of atropine-fast receptors may be related to the distribution of cholinergic fibers to vascular beds.

Summary. Data are presented which demonstrate that atropine blockade of cholinergic effects in vascular beds is reversible. The reversibility of the blockade is evident in the vasodilatation and blood pressure fall which result from postatropine injections of ACh. It appears to be related not to time, size of dose, or interval between doses, but to the number of times ACh is allowed to compete for the receptor site.

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