Buffer reflexes, tolerance to ganglioplegics and their relationship to enhanced pressor responsiveness

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Adminstration of ganglion blocking agents increases cardiovascular reactivity as measured by pressor-depressor responses to a variety of drugs (1). The suggestion has been made that this phenomenon is due entirely to loss of compensatory cardiovascular reflexes (2). One of the most important of these, the loss of which enhances arterial pressure responses, is the carotid sinus buffer mechanism (3-5).

Our objective was to determine whether the increase in reactivity that follows injection of ganglioplegics parallels inhibition of the carotid occlusion reflex. A close correlation would support the hypothesis that augmented responses to vasoactive agents following ganglion blockade depend largely, or entirely, upon loss of compensatory reflexes.

The problem became more complex when it was found that, while the carotid occlusion reflex was sharply inhibited following initial administration of one of the ganglioplegics, with time and further administration of the drug, arterial pressure and the reflex returned, both often nearly to the control values. Further dosage of ganglioplegic at this time failed to reduce either arterial pressure or the carotid sinus reflex. In short, a state of tolerance seemed to have developed that resembled the one described by Page, Del Greco and Corcoran (6) in dogs in which large doses of ganglioplegic failed to lower arterial pressure. We have, therefore, measured the degree of augmentation of pressor responsiveness both before and after appearance of tolerance to ganglioplegics and, employing electroneurographic techniques in part, attempted to determine the mechanism by which arterial pressure is maintained at normal levels and neurogenic compensatory reflexes remain operative in the presence of extremely high concentrations of ganglioplegic agents.

Methods

The development of tolerance to ganglioplegic agents in dogs is a variable phenomenon but usually may be elicited provided enough time is allowed, and enough tetraethylammonium chloride (TEAC) or other ganglioplegic given. Morphine (2 mg/kg) and pentobarbital (15 mg/kg) pentobarbital alone (30 mg/kg) or morphine (12 mg/kg) and chloralose (80-100 mg/kg) were used as anesthetics in dogs. Cats were anesthetized with 30 mg/kg of pentobarbital given intravenously or intraperitoneally; the vagus nerves were cut and the carotid arteries exposed. Test doses of synthetic angiotensin (7),

*Angiotensin is the name recently proposed by Braun-Menendez and Page (6) as a substitute for angioten in and hypertensin.
serotonin (60 or 120 gamma of base) or norepinephrine (5 or 10 gamma) were given into a femoral vein. Heart rate was recorded on a smoked drum by interrupting the vertical sweep of the recording arm of a Palmer drop recorder by the animal's amplified ECG. The unit was designed and constructed by Mr. Harold Nastelin. Articular pressure was recorded on the same smoked drum by a mercury manometer connected to a cannulated femoral artery. TEAC was administered intravenously in doses of 5–10 mg/kg until an average of 65 mg/kg had been given during 2 to several hours; an intravenous infusion of 60 mg/kg was given concurrently. Shortly after the administration of TEAC was begun, intermittent positive pressure respiration was initiated. Level anesthesia was maintained by intramuscular injections of small doses of pentobarbital.

Directly after giving TEAC, the carotid occlusion reflex was often sharply reduced but this was followed by rapid, partial return. For our purposes, true tolerance existed only when 10 mg/kg of TEAC intravenously had little and transient effect on the occlusion reflex. The TEAC time/dose relationship is of importance. Insofar as possible, sharp and prolonged falls in arterial pressure were avoided as they often prolonged the experiment unduly and prevented the giving of enough TEAC to elicit tolerance. The ganglion blocking agents tetraethylammonium chloride, mecamylamine (Iversine) chlorisondamine (Ecolid) or hexamethonium were all used but the greatest number of experiments was done with TEAC.

In other experiments on both dogs and cats, efferent activity in the postganglionic inferior cardiac and renal nerves was measured electroneurographically and correlated with blood pressure response to ganglioplegics and the tolerance phenomenon. The inferior cardiac nerve was found usually to arise from the inferior cervical sympathetic ganglion in dogs and from the stellate ganglion in cats. A renal nerve was dissected free from the surface of a renal artery. Using a dissecting microscope, a nerve was cut peripherally and the central end freed of connective tissue and sheath and then placed on silver or platinum wire electrodes connected to capacity coupled amplifiers. Electric activity was displayed on the face of a cathode ray tube and photographed on moving paper. Femoral or carotid arterial pressure was measured with a strain gauge manometer and recorded photographically from a cathode ray tube simultaneously with nerve activity.

**RESULTS**

Plots were made for each of 19 experiments on dogs in which different blocking agents were used and the pressor responses to carotid occlusion and to norepinephrine, angiotensin or serotonin each separately constituted the abscissa, and time the ordinate. Inspection showed that with repeated single injections of TEAC, mecamylamine or chlorisondamine, there was initial, prompt inhibition of the reflex while augmentation of response to norepinephrine, serotonin, natural or synthetic angiotensin appeared slowly. After the carotid occlusion response had reached a level at which it usually was no longer reduced, rapid further augmentation of response to both norepinephrine and serotonin occurred.

At the beginning of the experiments, administration of ganglioplegics sharply reduced or almost abolished the pressor response to occlusion of the carotid arteries. If administration of a ganglioplegic was continued, it no longer lowered arterial pressure and, especially in the case of TEAC, raised it. During this phase—often several hours after the initial dose—the pressor response to carotid occlusion began to return and might even reach the control value, though the time required for the response to become maximum was considerably prolonged. During the phase of tolerance, the response was usually of the order of 25 mm Hg less than the control value. On reappearance of the occlusion response, surprisingly, the augmented responses to pressor drugs persisted (fig. 1). As a test for true tolerance, a dose of 10 mg/kg of TEAC was given intravenously directly after measurement of the occlusion reflex. There should be but little and transient reduction in response on repeating the occlusion reflex. This state may be said to be one of tolerance to ganglioplegics and, in the experiments, occurred ordinarily after an average of 65 mg/kg of TEAC had been injected and 60 mg/kg infused. During it, the occlusion response and the augmented responses to pressor drugs were unaltered by atropine (1 mg/kg) or tetraethylammonium iodide (10 mg/kg) or by injection of further large amounts of one or several of the ganglioplegics. Cross tolerance to ganglioplegics of even different chemical structure was found. Tolerance occurred independently of whether pentobarbital alone or pentobarbital and morphine or chloralose and morphine was used as anesthetic.

Initial large doses of ganglion blocking agent did not completely eliminate the occlusion response. A small
residual rise (10–15 mm Hg) was apparently due to the
hemodynamic effect of eliminating a portion of the pe-
ripheral vasculature since it was diminished little, if at
all, by anesthetizing the brain stem with intracisternal
injection of 500 mg of procaine or 200 mg of xylocaine.

Occlusion of both common carotid arteries was accom-
pnied by marked increase in heart rate; after adminis-
tration of ganglioplegic agents the cardiac response
along with the pressor response was greatly reduced or
abolished. With development of tolerance to the gangli-
oplectic, the resting heart rate regularly showed progres-
sive slowing until it was one-third to one-half the control
value; the pressor response to carotid occlusion was usu-
ally accompanied by moderate increase in heart rate but
this was not a regular event. This chronotropic response
was, when present, usually small and did not parallel
augmentation of response to pressor drugs, implying that
the pressor response to carotid occlusion during tolerance
to ganglioplegics does not depend to a large degree upon
cardiac acceleration.

When large doses of ganglioplegics had effectively re-
duced the response to carotid occlusion, and some aug-
mentation of response to pressor agents had occurred,
prostigmine (1 mg) or a prostigmine analog and pseudo-
cholinesterase blocking agent, 2-hydroxy-5-phenylbenzyl
trimethylammonium bromide dimethyl carbamate (RO
2-683) (0.5–1.5 mg) given intravenously usually caused
a slow, prolonged rise of 20–50 mm Hg in arterial pres-
sure and increase in response to carotid occlusion—occas-
ionally to the control level. Simultaneously, the al-
ready augmented response to noradrenaline was either
unaltered or reduced. The antipseudocholinesterase was
ineffective in some experiments.

When tolerance had become established, prostigmine
in repeated doses of 0.5 mg caused rises of arterial pres-
sure of about 50 mm Hg which did not persist. With re-
turn of pressure to control levels, it was found that the
occlusion reflex was unchanged but, after half an hour,
tended to increase. Responses to norepinephrine and
angiotensin were first reduced but then tended to in-
duce the response to carotid occlusion, and some aug-
mented responses occurred after half an hour, and, in most experiments, a dampened carotid occlusion response persists.

Effect of TEAC on evoked potentials in the inferior cardiac
nerve. In 11 experiments on dogs a dampened carotid occlusion response persisted.

Effec of TEAC on spontaneous electric activity in the inferior
cardiac nerve. In three cats and two dogs, spontaneous ac-
activity was measured from the cut central end of the left
inferior cardiac nerve. Normal activity in this postga-
ganglionic nerve was pulsative and was respiratory
modulated. Total activity increased sharply during the
response to carotid occlusion and disappeared tran-
siently with release of the carotid clips (fig. 2). In all
experiments, 5 mg/kg of TEAC intravenously caused
prompt, complete disappearance of nerve activity and
recovery of activity lagged significantly behind recovery
of arterial pressure. In one experiment on a dog, nerve
activity did not return after a single injection of 5 mg/kg
of TEAC despite persistence of a moderate carotid oc-
closure response. Much later, there was return of a small
amount of activity and this was again eliminated by
TEAC. In the other two experiments on cats and in two
experiments on dogs there was progressively less re-
covery of nerve activity after each dose of TEAC until,
after from three to five doses of 5 mg/kg, activity was
essentially gone, yet recovery of arterial pressure oc-
curred after each injection and, in most experiments,
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Figure 2: Top trace of each strip carotid arterial pressure. Bottom trace is electric activity in cat's left inferior cardiac nerve. Breaks in top reference line at 1-sec. intervals. A: carotid occlusion with release at signal line at bottom; B and C: continuous record with 5 mg/kg TEAC given i.v. during time indicated by signal line; D: carotid occlusion 20 min. after B and C. Pentobarbital anes-
thesia.
tic transmission. In three experiments in which TEAC caused complete blockade of both occlusion response and synaptic transmission, prostigmine caused return of the occlusion response but not of activity in the inferior cardiac nerve.

As noted by Schneider and Moore (9) a more rapid rate of stimulation caused increased susceptibility to ganglionic blockade. With recovery of transmission following intravenous TEAC, increase of stimulation rate from once every 5 seconds to 1 or 2/sec. caused sharp diminution or disappearance of the synaptic potential.

Brief tetanic stimulation of the preganglionic chain after complete blockade would, in most experiments, restore synaptic transmission either wholly or partly when the nerve was subsequently stimulated again at 5-second intervals. The effect was variable, often of short duration, and another injection of TEAC would again block transmission for a prolonged period of time, or until tetanic stimulation was repeated.

**Effect of TEAC on spontaneous efferent activity in renal nerves.** In six experiments on dogs and in five on cats, one or more small nerves were freed from on or near the surface of the left renal artery, cut peripherally and electric activity recorded from the central ends. Dissection at the end of the experiment usually showed the nerve to arise from the celiac ganglion; the origin of other nerves was from a paravertebral sympathetic ganglion at the level of the renal artery. The pattern of electric activity, like that in the inferior cardiac nerve, was pulse and respiratory modulated and total activity increased sharply during the response to carotid occlusion (fig. 3), indicating its probable vasomotor nature. With release of the carotid arteries or during pressor responses to intravenously injected norepinephrine, there was transient inhibition or elimination of electric activity. These procedures were employed in each experiment to ensure that the recorded activity was probably concerned with sympathetic control of the peripheral vasculature. A single dose of 5 or 10 mg/kg of TEAC caused complete disappearance of activity in all experiments, (fig. 3) but, unlike the rapid disappearance of electric activity in the inferior cardiac nerve, there was often a relatively long delay before blockade was complete.

Susceptibility to synaptic blockade varied widely among experiments and different nerves in the same experiment, whether on cats or dogs. There was variation not only in latent period before beginning of blockade, but in time required for blockade to become complete, time at which recovery of nerve activity began, and the degree of maximum recovery. Resistance to blockade by some nerves was the chief difference in results obtained from renal compared with inferior cardiac nerves—which showed quite prompt, complete disappearance of activity in all experiments after a single injection of TEAC. Additionally, changes in activity in renal nerves were sometimes only remotely related to changes in arterial pressure due to injection of TEAC. In one experiment on a cat, for example, activity in the first nerve tried was not changed immediately by TEAC despite a large fall in arterial pressure; blockade became complete only after 7 or 8 minutes and, at 12 minutes, when arterial pressure had largely recovered, no nerve activity was recordable. At this time, a second nerve running parallel with the first one showed a large amount of activity that was eliminated very promptly by a second injection of TEAC.

It was difficult to elicit tolerance to TEAC in these experiments; this may have been due to some deterioration of the preparation during the extensive dissections required for adequate exposure. A degree of tolerance appeared in several experiments however. In the others, successive injections of TEAC usually continued to cause falls in arterial pressure and there was less return of nerve activity after each injection, despite good or full recovery of arterial pressure. Compared with response of the inferior cardiac nerve, however, activity tended to...
persists to some degree after a greater number of injections of TEAC and the small amount present after several injections would still increase during a dampened carotid occlusion response.

In those experiments in which tolerance to TEAC could be elicited, nerve activity persisted in some nerves in each experiment but was invariably minute when compared with normal activity present before giving TEAC, and this small amount of activity was often not so clearly pulse and respiratory modulated. It was difficult to record but appeared definite, and would still show a small increase during carotid occlusion (fig. 3), implying its continued vasomotor nature and tending to exclude injury or other random activity. To ensure that the recorded activity was not a potential picked up from another source, such as a twitching muscle, or due to motion of the nerve upon the electrodes, a drop of procaine was applied to the end of the nerve which eliminated all activity.

During partial tolerance to TEAC, different nerves displayed different degrees of susceptibility to blockade. While small amounts of nerve activity could be recorded from some nerves, none could be recorded from others. That elimination of activity was due to TEAC rather than trauma or deterioration of the preparation was indicated by the observation that prostigmine, given intravenously, caused increased activity in already active nerves and caused activity to appear in previously inactive ones. In essentially all experiments, some susceptibility to blockade by TEAC persisted throughout, and the carotid occlusion responses recovered to only a fraction of their control values. This relative incomplete state of tolerance may have accounted in part for the small amounts of recordable nerve activity.

**DISCUSSION**

Ganglioplegic agents are well known to block the pressor effect of carotid occlusion but, as we have noted previously (16), after section of the vagus-sympathetic-depressor nerve trunks, and in the presence of high concentrations of TEAC, cutting the carotid sinus nerves is followed by a large and prolonged rise in arterial pressure and further increase in the already augmented response to angiotensin. Clearly, the buffering capacity of these reflexes had not been abolished wholly by the ganglioplegics.

We have, therefore, studied more extensively the response to carotid occlusion under different experimental conditions to define any relationship it might have to the augmentation of response to pressor drugs that follows administration of ganglion blocking agents. Since the buffer reflexes are important in the compensatory regulation of arterial pressure, and since their elimination has been shown to enhance responsiveness to pressor drugs (3-5) it was reasonable to suppose that their progressive suppression by ganglion blocking agents would be paralleled by a linear increase in pressor responsiveness. But this was not the case.

The occlusion response was usually lost well before full augmentation of response to pressor drugs appeared.

With time and repeated administration, the ganglioplegics lost their ability to prevent the occlusion response, yet augmented responses to pressor agents persisted and, further, augmentation was usually greater at this time than when the carotid occlusion response had been largely inhibited early during the experiment.

These results suggest that at least two major mechanisms account for the augmented responses to pressor agents that follow administration of a ganglioplegic drug. First, as has been shown (3-5), elimination of the carotid sinus and aortic buffer mechanism results, logically, and in fact, in larger responses. But persistence of augmented responses after ganglia become refractory to ganglioplegics, and after the carotid occlusion response has been restored by administration of a cholinesterase inhibitor, and the further augmentation by ganglioplegics after sectioning all four buffer nerves (10) all indicate that another mechanism is also involved. It may be a peripheral vascular sensitization allied to that which follows denervation. Once augmentation occurred, it usually persisted, largely independent of functional activity of the buffer reflexes.

Assuming there is peripheral vascular sensitization after administration of ganglioplegics to the point of tolerance, reflex responses to carotid occlusion may depend upon a very few impulses getting through sympathetic ganglia with increased sensitivity of the arterioles to a smaller amount of humoral mediator accounting for responses of normal or near-normal magnitude. While electroneurographic measurements did not reveal such impulses in postganglionic inferior cardiac nerves, measurements from postganglionic renal nerves demonstrated impulses in some nerves in some experiments. While the amount of recordable activity was exceedingly small, and not demonstrable in all nerves, it changed characteristically with reflex or drug induced changes in arterial pressure. By and large, the greater the total amount of TEAC administered, the smaller the amount of nerve activity associated with maintenance of resting pressures and the pressor response to carotid occlusion. While these observations are compatible with a theory of peripheral sensitization by ganglioplegics, they are not proof that the phenomenon occurs. Supporting evidence for the theory derives from the observation that sensitivity of response to injected noradrenaline increases with time and the continued administration of ganglioplegic.

Should the continued presence of the carotid occlusion response after administration of large amounts of ganglioplegic depend upon a mechanism of peripheral sensitization, the nerve impulses responsible are apparently not transmitted over all sympathetic pathways. Not only were they not observed in the inferior cardiac nerve but they did not appear in some renal nerves. Too, evoked potentials in the inferior cardiac nerve due to stimulation of the sympathetic chain at T2 or T3 were eliminated by ganglioplegics despite the continued...
presence of a carotid occlusion response. Measurements of heart rate throughout experiments in which tolerance to ganglioplegics developed also imply that the cardio-accelerator pathways, at least, remain blocked. While a small increase in heart rate was often seen during the occlusion response at this time, it may have depended upon release of endogenous epinephrine or norepinephrine from other sites in the body. The existence of cardiac nerves not susceptible to blockade by TEAC was shown by Pardo, Rennick and Moe (11) but these pathways to the A-V node were not activated by carotid occlusion in their experiments.

While the carotid occlusion reflex may be comparable in magnitude to control responses following continued administration of large amounts of ganglioplegics, the form of the response is changed. Normally the rise in pressure is rapid, a maximum value being reached within 15 or 20 seconds. During tolerance the rise is much slower and 2 or 3 minutes may elapse before it is maximal, and the response persists longer after releasing the carotid arteries.

Large amounts of the ganglioplegic drugs were required to reduce sharply, or abolish, the carotid occlusion reflex and often the reduction lasted only a matter of 10-40 minutes before it began gradually to return, accompanied by tolerance. Further repeated large doses (i.e. 10 mg/kg of TEAC) failed to block the reflex. Since, under the circumstances, little of the drug is excreted, since it is not metabolized significantly, and since it is given by continuous infusion, it may be assumed that blood levels are consistently high. TEAC competes with acetylcholine for the receptors of the ganglion cell membrane. The combination of TEAC with receptor substance may fail to initiate the general increase in membrane permeability to ions followed by depolarization, which is believed to be the sequence of events leading to stimulation (12). Or, even if the combination did so, repolarization may occur and yet the block persist, if there is an analogy with the neuromuscular block elicited by acetylcholine in isolated frog skeletal muscle.

Thesleff (13) showed that here depolarization caused by acetylcholine itself lasted only 10-15 minutes but block persisted when the resting membrane potential was completely repolarized.

If we assume that TEAC-receptor substance fails to initiate response as does the acetylcholine combination with receptor substance then there are at least two possible explanations for a return of nerve transmission during tolerance: a) since the body is unable to destroy TEAC, the continued blockade of acetylcholine receptors by TEAC leads to the use of alternative chemical mediators. b) TEAC may fit acetylcholine receptors less well than acetylcholine itself so that even a moderate increase in concentration of acetylcholine increases the chance of combination with free receptors. Possible support for this point of view comes from Maxwell et al. (14) who found that large doses of ganglioplegics failed to prevent pressor responses to supramaximal faradic stimulation of a splanchnic nerve. In the experiments reported here, brief tetanic stimulation of the preganglionic nerve was found to restore seemingly normal synaptic transmission after it had been blocked by TEAC. The anticholinesterase activity of TEAC (15) may also be a factor in the buildup of concentration of acetylcholine at the ganglion synapse sufficient to compete successfully for the receptor sites.

Tolerance to ganglioplegics also develops in hypertensive patients under treatment for long periods of time in that these agents fail then to lower arterial pressure (16, 17). Patients differ greatly in the speed and degree of tolerance developed. Parasympathetic neural transmission is probably restored in part since blurring of vision, dryness of the mouth, difficulty in micturition and decreased bowel motility are gradually corrected. During this state, even intravenous administration of more ganglioplegic fails to lower arterial pressure or to make the symptoms of parasympathetic paralysis reappear.

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