SUPPLEMENTARY STUDIES ON THE SITES OF ACTION OF PILOCARPINE

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Although it is generally accepted that pilocarpine affects the postganglionic cholinergic receptors and reveals a strong muscarinic action selectively, evidence has been cited to conclude that the drug affects other sites of the effector organs. Large doses of the drug do not induce a rise of blood pressure in an atropinized animal. But Dale and Laidlaw (1), Marrazi (2) and Ambache (3) concluded that pilocarpine stimulated the superior cervical ganglion in the cat, based on the experimental results that the drug potentiated the contraction of the nictitating membrane and the postganglionic electrical potential in response to preganglionic sympathetic stimulation. Feldberg et al. (4) proved that pilocarpine induced a discharge of adrenaline from the adrenals. The authors all agreed that the ganglion stimulating action of pilocarpine was abolished after the administration of atropine in large doses.

The evidence that pilocarpine induced a strong pressor action on the blood pressure after the administration of such ganglion blocking agents as nicotine (5), d-tubocurarine and tetraethylammonium (6), hexamethonium, laurifoline and menisperine (7) raises the question regarding other sites of the action of pilocarpine.

Another question to be investigated is raised by the evidence that in the course of recovery from the augmenting effect of pilocarpine the salivary response of the submaxillary gland to stimulation of the chorda tympani was depressed (8).

Lastly, there is the problem of the innervation and the response of the sweat glands to nerve stimulation. The sweat glands are innervated by the sympathetic postganglionic fibers, but they respond not to adrenergic but to cholinergic drugs.

These former three questions were studied in the present investigation to clarify the mechanism and the site of the action of pilocarpine.

METHODS

Cats, weighing 1.5 to 2.0 kg and dogs, weighing 5 to 15 kg, were used.

1) Superior cervical ganglion of the cat

Cats, under evipan sodium anesthesia (50 mg/kg intraperitoneal) or spinal cats, sectioned between C I and C II under ether anesthesia were used. The electrical stimulation of the pre- or postganglionic fiber of the cervical sympathetic nerve and the recording of the contraction of the nictitating membrane in response to stimulation followed the method used by Inoue (7).
2) Arterial pressure in the dog

Blood pressure from the femoral artery of the dog under amytal sodium anesthesia (50 to 70 mg/kg intraperitoneal) was recorded in the usual method.

3) Salivary flow of the submaxillary gland in the dog

The salivary flow of the submaxillary gland in response to stimulation of the chorda tympani and to pilocarpine was recorded following the method which Shimamoto and Inoue (8) used. The cervical sympathetic and splanchnic nerves were prepared for stimulation when required.

4) Drugs

Pilocarpine hydrochloride; as ganglion blocking agents hexamethonium bitartrate, menisperine chloride and laurifoline chloride; as sympathomimetics l-adrenaline hydrochloride and dl-noradrenaline hydrochloride; as adrenolytics dibenamine, priscol and chlorpromazine; as a parasympatholytic atropine sulfate were used. The drugs were injected into the femoral vein.

RESULTS

I. Nictitating Membrane of the Cat

1) Action of pilocarpine on the normal and denervated nictitating membrane (Fig. 1)

The administration of pilocarpine above doses of 20 to 50 μg/kg contracted the normal and the denervated nictitating membrane of the cat. The response of both membranes to 200 μg/kg of pilocarpine corresponded to the response to 5 μg/kg of adrenaline. The response to 50 μg/kg of pilocarpine plus 5 μg/kg of adrenaline was more effective than to the single administration of each drug. The response to pilocarpine was sensitized by the chronic denervation of the superior cervical ganglion. The contractive effect of pilocarpine on the membrane
was abolished after the administration of 0.1 mg/kg of atropine for a long while, but the contractive effect of adrenaline or noradrenaline was only depressed temporarily. The response of the membrane to these drugs were similarly potentiated after the administration of hexamethonium, laurifoline and menisperine, each in the ganglion blocking dose. The response of the normal and denervated membrane to pilocarpine and to cervical sympathetic stimulation were slightly depressed after the administration of priscol or dibenamine in full adrenolytic doses (10 to 20 mg/kg), while the same response to adrenaline or noradrenaline was markedly depressed or abolished by the same treatment. Moreover it was observed that the response of the membrane to cervical sympathetic stimulation was abolished by the administration of dibenamine in an atropinized animal in which pilocarpine did not exhibit any effect.

2) Effect of pilocarpine on the contraction of the nictitating membrane in response to preganglionic sympathetic stimulation

One hundred to 200 μg/kg of pilocarpine potentiated slightly the response of the nictitating membrane to preganglionic cervical sympathetic stimulation, as illustrated in Fig. 2. After

\[ S \text{ : Electrical stimulation, submaximal, } 10/\text{sec} \]

the administration of 0.1 mg/kg of atropine, not only the contractive effect but also the potentiating effect on the nerve stimulation was abolished. The potentiating effect of pilocarpine was also abolished after the administration of hexamethonium, laurifoline and menisperine in doses of 1 to 2 mg/kg (Fig. 3).

3) Effect of pilocarpine on the contraction of the nictitating membrane in response to postganglionic sympathetic stimulation

The administration of over 300 μg/kg of pilocarpine potentiated the response of the membrane postganglionic cervical sympathetic stimulation. The potentiating effect of pilocarpine was also abolished by atropine in doses of 0.1 mg/kg, but the same effect was only depressed after the administration of priscol or dibenamine in the adrenolytic dose.
FIG. 3. Effect of hexamethonium, dibenamine and atropine on the response of the nictitating membrane to pilocarpine.

S: Preganglionic stimulation of the cervical sympathetic nerve, submaximal, 10/sec

4) Effect of atropine and dibenamine on the response of the nictitating membrane to pre- and postganglionic stimulation of the cervical sympathetic nerve

The response of the nictitating membrane to preganglionic cervical sympathetic stimulation was almost equally depressed after the administration of 1 to 2 mg/kg of atropine or 10 to 15 mg/kg of dibenamine. As illustrated in Fig. 3 and 4 the response of the membrane...
to pre- or postganglionic stimulation of the cervical sympathetic nerve was depressed about by half by dibenamine. The remaining response of the membrane to nerve stimulation was abolished by the administration of 1 to 2 mg/kg of atropine. In the case of reversed procedure of administration, a similar effect was also observed. Even when the amounts of each drug was increased, the depressive effect of the drugs on the membrane did not increase further.

II. Blood Pressure in the Dog

1) Effect of pilocarpine

The administration of 20 to 50 \( \gamma/\text{kg} \) of pilocarpine showed a striking fall of arterial pressure with a subsequent rise. The response, especially the subsequent rise was subjected to a good deal of individual variation. The secondary rise of blood pressure in response to 50 \( \gamma/\text{kg} \) of pilocarpine was usually extended to 5 to 10 mm Hg.

2) Effect of stimulation of the splanchnic nerve on the action of pilocarpine

During continued stimulation of the splanchnic nerve, by which the blood pressure was inclined to rise, the effect of pilocarpine was compared with the control. The result, as illustrated in Fig. 5, showed that the stimulation of the nerve depressed the fall and markedly potentiated the secondary rise of blood pressure in response to pilocarpine.

![Fig. 5. Effect of the splanchnic stimulation on the response of blood pressure of the dog to pilocarpine. S : Splanchnic stimulation, submaximal, 10/sec](image)

3) Effect of adrenaline and noradrenaline on the action of pilocarpine

The pressor effect of 2 to 5 \( \gamma/\text{kg} \) of adrenaline or noradrenaline was little affected by 50 to 100 \( \gamma/\text{kg} \) of pilocarpine. The depressor response to pilocarpine was slightly depressed by the continued infusion of adrenaline at the rate of 0.5\( \gamma/\text{kg/min} \).

4) Effect of the ganglion blocking agents on the action of pilocarpine

After the administration of such ganglion blocking agents as hexamethonium, menisperine or laurifoline, each in a dose above 50 \( \gamma/\text{kg} \), the depressor effect of pilocarpine was markedly
inhibited or abolished, while the pressor effect was markedly augmented in height and duration of action. These effects were similarly observed in the bilateral adrenalectomized and also in spinal animals as well as in the intact animals (Fig. 5 and 6). The marked pressor effect of pilocarpine was also observed in the course of continued infusion of the ganglion blocking agents at the rate of 100 γ/kg/min.

5) Effects of atropine on the pressor action of pilocarpine

The administration of 0.1 to 1.0 mg/kg of atropine abolished not only the depressor effect, but also the secondary pressor effect and the marked pressor effect of pilocarpine by the ganglion blocking agents.

6) Effect of adrenolytics on the action of pilocarpine

The marked pressor effect of pilocarpine, sensitized by the ganglion blocking agents, was abolished by the administration of such adrenolytics as dibenamine or priscol in an adrenolytic dose. But chlorpromazine did not abolish the pressor effect of pilocarpine even in an adrenolytic doses. It was noticed that some of the derivatives of chlorpromazine, which sensitized the action of adrenaline, depressed or abolished the pressor action of pilocarpine.

III. Secretory Response of the Submaxillary Gland in the Dog

1) Effect of pilocarpine

The threshold dose of pilocarpine to induce a submaxillary secretion was subjected to a good deal of individual variation. The response of the sympathetic denervated gland to pilocarpine was stronger than that of the normal gland. But it was often observed that the duration of the salivary effect of pilocarpine on the denervated gland was shorter than on the normal gland. In the course of the response of both glands to pilocarpine, the saliva secreted became more and more viscous in association with the reduction of secretion. The response of the gland to pilocarpine showed a seasonal variation. In winter the saliva secreted was highly viscous and the gland was less sensitive to pilocarpine.
2) Effect of pilocarpine on the response of the submaxillary gland to stimulation of the chorda tympani

Pilocarpine also potentiated the salivary response of the submaxillary gland to stimulation of the chorda tympani. The threshold dose of the drug was 5 to 10 \( \gamma/\text{kg} \). In the course of the response of the gland to 30 to 50 \( \gamma/\text{kg} \) of pilocarpine it was often observed that the salivary secretion showed a relative decrease for a while following the potentiating effect. The depressive effect on salivary secretion accorded with the secondary pressor effect of pilocarpine. The duration of the potentiating effect of a definite dose of pilocarpine on the salivary secretion induced by stimulation of the chorda tympani was usually shorter than the duration of the secretory effect of the drug in the same dose.

3) Effect of the ganglion blocking agents on the response of the submaxillary gland to pilocarpine

In the course of the salivary secretion induced by pilocarpine, the administration of hexamethonium, laurifoline and menisperine depressed or abolished the secretion. Intensity of the depressive effect by the drugs depended on the dose administered. After administration of the ganglion blocking agents in doses sufficient to abolish the submaxillary response to stimulation of the chorda tympani, pilocarpine showed two types of effect. The one was a depressive effect which was usually observed, and the other was either no effect or a somewhat potentiating effect. The latter effect was often seen in summer.

4) Effect of adrenaline or cervical sympathetic stimulation on submaxillary secretion by pilocarpine

Adrenaline or cervical sympathetic stimulation depressed the salivary secretion of the gland induced by stimulation of the chorda tympani. As mentioned above, the submaxillary secretion was first potentiated and then slightly depressed by pilocarpine. In the course of the depressive or recovering phase to pilocarpine action, the depressive effect of adrenaline or of sympathetic stimulation appeared more marked than before treatment with pilocarpine.

DISCUSSION

The present experiments were designed to settle the question of the action of pilocarpine, other than the muscarinic effect and the site of action. In spite of the general acceptance that the nictitating membrane of the cat is innervated only by sympathetic adrenergic postganglionic fibers (9), Bacq and Frederiq (10) claimed that parasympathetic cholinergic fibers were also present. The contractive response of the membrane to acetylcholine (1, 11, 12) was explained by the liberation of sympathin from the adrenals and by the stimulation of the superior cervical ganglion.

The result in the experiments here demonstrated the following points in regard to the innervation of the membrane. The response of the membrane to stimulation of the pre- or postganglionic fibers of the superior cervical ganglion was depressed about by half by atropine or dibenamine. The response of the membrane was completely abolished by the combined administration of either drug.

It was reported that such adrenolytics as dibenamine (13, 14) or chlorpromazine (15-17) blocked the response of the membrane to adrenaline but only depressed the same response
to electrical stimulation of the pre- or postganglionic cervical sympathetic nerve. The latter
evidence was explained as being due to the liberation of noradrenaline which was more resistant
to adrenolytics than adrenaline (18) or to the differential response of the membrane to circu-
lating adrenaline and to the endogenous adrenaline (14). The results of the present experi-
ments showed that after the administration of atropine, the response of the membrane to ad-
renaline and to sympathetic stimulation was equally blocked by dibenamine. From this
evidence it was concluded that the nictitating membrane of the cat was innervated by both
adrenergic fibers. Other evidence to support the conclusion was described later concerning
the action of pilocarpine.

Pilocarpine contracted the nictitating membrane of the intact, spinal, superior cervical gan-
glionectomized and adrenalectomized cat. The contraction in response to 100 μg/kg of pilocarpine
 corresponded to the response to 0.2 to 0.5 μg/kg of adrenaline. Adrenalectomy or superior cer-
vical ganglionectomy depressed but not abolished the response to pilocarpine. From these
results we came to the conclusion that pilocarpine effected the postganglionic receptor of the
membrane. The contractive effect of pilocarpine was blocked by 0.1 mg/kg of atropine. The
response of the membrane to pilocarpine was relaxed but not blocked even by a large dose
of dibenamine. The contraction of the membrane by adrenaline was potentiated by the pre-
treatment with pilocarpine. But the potentiating effect of pilocarpine on the response to
adrenaline was abolished by atropine as well as by dibenamine. This evidence supports the
conclusion that pilocarpine effects the postganglionic adrenergic receptor of the membrane.

The fact that pilocarpine, in doses insufficient to contract the membrane, potentiated the
response of the membrane to preganglionic stimulation of the cervical sympathetic nerve,
and that the response of the intact membrane to preganglionic stimulation was potentiated
only by large doses of pilocarpine, support the assumption of the ganglion stimulating effect
of the drug which Dale and Laidlaw detected. In one of our experiments, 100 μg/kg of pilo-
carpine relaxed the normal membrane, while contracted the acutely denervated membrane
in the adrenalectomized animal. This evidence indicates that the ganglion stimulating effect
of the drug was, if it existed, weaker in comparison to the postganglionic effects.

It was reported that pilocarpine induced a secondary rise of blood pressure (19), which
was blocked by atropine (20). It was also stated that the response was depressed or aboli-
shed by adrenalectomy. In this experiment adrenalectomy did not induce any significant
change of the action of pilocarpine, which agreed with Root. After the administration of such
ganglion blocking agents as hexamethonium, laurifoline or menisperine, pilocarpine caused
a marked pressor effect. Root proved that the effect of pilocarpine by tetraethylammonium
was neither modified by adrenalectomy or spinal cord section and was depressed by atropine,
ergotoxine, dibenamine or C-7337. Similar results were also obtained in these experiments.
Adrenolytics as well as atropine blocked the pressor effect of pilocarpine, while adrenolytics
unlike atropine did not effect or slightly depressed the depressor effect of pilocarpine. Therefore
it was supposed that pilocarpine affected the postganglionic adrenergic receptors, thereby in-
ducing the rise of blood pressure. The marked pressor effect of pilocarpine after the adminis-
tration of the ganglion blocking agents depends upon a similar mechanism that the pressor
effect of adrenaline or noradrenaline was modified by the blockades (21). Besides it was noticed
that the muscarinic and adrenergic action of pilocarpine were selectively antagonized by atro-
pine. Further, it might be mentioned that chlorpromazine which was a potent adrenolytic,
proved to have a weak antagonizing effect, while some of the derivatives of chlorpromazine
which were sympathomimetics, proved to have moderate antagonizing effects on the pressor
action of pilocarpine (22). The rise of blood pressure in response to pilocarpine was potentiated
during the continued infusion of adrenaline or the continued stimulation of the splanchnic
nerve. These results also supported the conclusion that pilocarpine affects the postganglionic
adrenergic receptors.

The administration of pilocarpine a profuse submaxillary secretion. Concerning the sali-
vary effect of pilocarpine, the following points were noticed. 1) Although the secretory
response of the sympathetic denervated glands were stronger than those of the normal glands,
the duration of the activity of the denervated structures was rather shorter than that of normal
glands. 2) The saliva secreted in response to pilocarpine became more and more viscous in
accord with the reduction of salivary flow. This resembles the salivation from the glands
sympathetically stimulated. 3) The salivary response to pilocarpine was less in winter while
the depressive effect of adrenaline or of cervical sympathetic stimulation on the cholinergic
induced salivation was marked. 4) In the recovering or the depressive phase of pilocarpine
action the depressive effect of adrenaline or of cervical sympathetic stimulation was mani-
fested stronger than in normal secretion in response to stimulation of the chorda tympani.
5) Ganglion blocking agents usually depressed the response to pilocarpine. But sometimes,
especially in summer, when the depressive effect of adrenergic stimuli was markedly weak,
the drug potentiated the salivary effect of pilocarpine. These evidences all confirmed the fact
that pilocarpine has a postganglionic action similar to the effect of adrenaline or sympathetic
stimulation.

CONCLUSION

The actions and the sites of pilocarpine other than the muscarinic effect were studied in
the nictitating membrane of the cat, blood pressure in the dog and the submaxillary secre-
tion in the dog. From the results obtained it is concluded that pilocarpine has three sites
and types of action, i.e. muscarinic, ganglion stimulating and adrenergic.

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