The Effect of Age on Adrenergic and Cholinergic Salivation (36554)

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We have previously shown (1, 2) that a stimulant barbiturate, sodium 5-(1,3-dimethylbutyl)-5-ethyl barbiturate (DMBEB), causes jumping activity which is greater in 1-month- than in 9-month-old mice. The jumping activity produced by this barbiturate is reduced or abolished by beta adrenergic and ganglionic blocking agents and is enhanced by *d*-amphetamine. These findings suggested that the jumping activity requires the functional integrity of the sympathetic nervous system. The decreased jumping activity induced in old mice by DMBEB suggests that the functioning of the sympathetic nervous system declines with age. As a further test of this concept, we have compared adrenergic and cholinergic salivation in 1-month- and 9-month-old mice.

Methods and Materials. NIH male mice 1 month (15–22 g), 6 months (32–38 g) and 9 months (25–40 g) old were used in these experiments. All injections were given intraperitoneally (ip). Salivation was graded (0, 1, 2 and 3) by visual observation of the extent of moistening of the lips, jaws, and chest, as previously described (3). The salivation for each mouse was recorded every 5 min for the first 30 min after injection of a sialogogue. The magnitude of the sialogogue effects was expressed as the sum of the successive gradings, with a possible range of 0–18.

In some experiments, salivation was induced in mice which were placed for 15 min in a box, the temperature of which was thermostatically set at 41–42°. Salivation was graded immediately after removal of each mouse from the box. Since only one grading was made, salivation scores could not exceed 3.

The data in Fig. 1 were subjected to an analysis of variance. The treatment means were tested for significant differences by Scheffe's method of multiple comparisons (4). The data represented in Fig. 2 were tested by Student's t test. For the responses to *d*-amphetamine where two comparisons were made, a difference was not considered significant ($p < 0.05$) unless the calculated value of t exceeded the value corresponding to $p = .025$. This precaution was taken because an apparently significant result is more likely to occur when several comparisons are made at the same nominal significance level. With k such comparisons, the use of $a = 1 - (1-a)^{1/k}$ guarantees significance at the a level (5).

The drugs used in these experiments included *d*-amphetamine sulfate and pilocarpine nitrate (K & K Labs., Plainview, NY), cocaine hydrochloride (Merck & Co., Rahway, NJ), and *l*-norepinephrine bitartrate (Winthrop Lab., Special Chemicals Dept., New York, NY). All doses are expressed in terms of the base.

Results and Discussion. The sialogogue response to *d*-amphetamine was significantly greater in 1-month- than in 9-month-old mice, when evaluated either in terms of percentage of mice salivating or in terms of cumulative salivation scores (Fig. 1). Almost all 1-month-old mice salivated after *d*-amphetamine, in sharp contrast to less than one-third of the 9-month-old mice. The salivation scores of the young mice were almost 6 times those of the 9-month-old mice. The

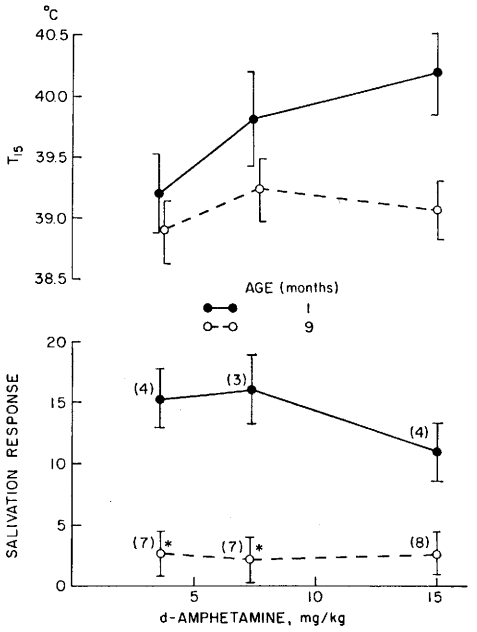

FIG. 1. Dose–response relationships for salivation responses and T_{15} values induced by the ip administration of *d*-amphetamine in young (1 month old) and old mature (9 month old) male mice. The T_{15} values are the body temperatures measured 15 min after the injection of *d*-amphetamine. The points represent the means ± SE of measurements on the number of mice indicated in the parentheses. An asterisk indicates that the value in old mice is significantly less than the corresponding value in young mice ($p < .05$).

salivation responses of 6-month-old mice were approximately one-third less than those of the young mice (Fig. 2).

The salivary glands of both young and old mice were capable of responding to both adrenergic and cholinergic sialogogues, as shown by the occurrence of similar responses in both groups to *l*-norepinephrine (after cocaine) and to pilocarpine (Fig. 2). All mice salivated in response to these agonists, regardless of age. We have previously reported that the dose of *l*-norepinephrine used in these experiments (0.5 mg/kg) did not produce visually observable salivation when administered alone (3).

Body temperatures 15 min after the injection of *d*-amphetamine (T_{15}) were lower in both 6- and 9-month-old mice than in 1-month-old mice (Fig. 2). In contrast, T_{15} temperatures after *l*-norepinephrine plus cocaine or pilocarpine were not lower in older mice (Fig. 2).

Since aging did not influence the responses of the salivary glands to a peripherally acting adrenergic drug (*l*-norepinephrine after cocaine), the receptor mechanisms in the gland cannot account for the differences in response to *d*-amphetamine in young and older mice. Perhaps *d*-amphetamine, an indirectly acting sympathomimetic amine, is not as effective in older mice because the release mechanism may decline with age, possibly due to the unavailability of norepinephrine in the nerve endings. Shibata *et al.* (6) have

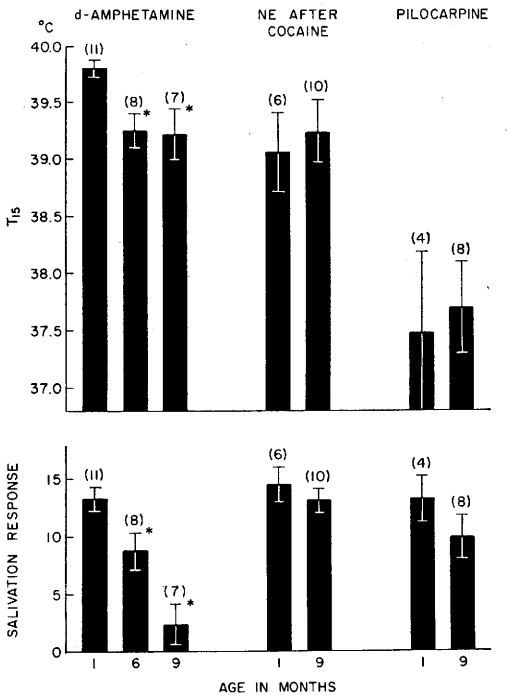

FIG. 2. The first three bars on the left represent salivation responses and T_{15} values for *d*-amphetamine (7.3 mg/kg) in mice 1, 6, and 9 months old. The two middle bars represent salivation responses and T_{15} values for *l*-norepinephrine (0.5 mg/kg) 5 min after cocaine (18 mg/kg), in 1- and 9-month-old mice. The two bars on the right represent salivation responses and T_{15} values for pilocarpine (15 mg/kg) in 1- and 9-month-old mice. Bars represent mean values ± SE for the number of mice indicated in parentheses. An asterisk indicates that the value differs significantly from that in 1-month-old mice ($p < .05$).

reported that the norepinephrine content of aortic strips from old (more than 10 months) male rabbits was significantly less than in young (5–6 weeks) rabbits. They also found that the sensitivity of aortic strips from young animals were more sensitive to the indirectly acting sympathomimetic amine, tyramine, than those from old rabbits (6). We have previously reported (7) that salivation responses to *d*-amphetamine were absent 4 hr after a large dose of reserpine, when the norepinephrine content of peripheral tissues is virtually depleted. According to Gutmann (8), muscle atrophy in old age is accompanied by a decrease of transmitter (acetylcholine) release and unchanged responses to this chemical. Responses to pilocarpine, a peripherally acting cholinergic sialogogue, are not affected significantly by age.

Summary. There is a marked decrease with age in salivation responses to *d*-amphetamine, accompanied by a reduced hyperthermic effect. Aging does not affect salivation responses or temperature effects produced by the directly acting adrenergic sialogogue, *l*-norepinephrine potentiated by cocaine, and by the cholinergic sialogogue, pilocarpine.

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