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Age- and Strain-Related Differences in Metabolic Response to Tyramine in Rats (41505)

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Abstract. Differences in metabolic response to intraperitoneally injected tyramine were examined in young (3–4 month) and old (20–24 month) Sprague–Dawley and Fischer 344 rats. Fischer 344 rats were found to be significantly more sensitive to tyramine than Sprague–Dawley. Optimal doses were 2 mg/kg body weight for young F344, 20 mg/kg for young Sprague–Dawley, 5 mg/kg for old F344, and >40 mg/kg for old Sprague–Dawley. Significantly higher doses were required by old rats than young. Mechanisms of these age- and strain-related differences in response are discussed. This information could well be important in selecting appropriate ages and strains of animals for experimental use, especially in aging research.

Tyramine, a naturally occurring sympathomimetic amine, has been identified as a normal constituent of some mammalian tissues (1). Exogenously administered tyramine is actively taken up by the sympathetic nerve endings into the catecholamine storage vesicles displacing catecholamines, primarily norepinephrine (NE), in the process (2). There are no apparent signs of toxicity when tyramine is employed, and the elevated metabolism following injections of either tyramine or NE is equivalent (3). In environmental physiological research tyramine has supplanted the use of NE as an indicator of the level of cold acclimation (3–6).

There is some information suggesting that the function of the adrenosympathetic nervous system is modified consequent to the aging process (7–11). There are two primary questions: (a) do older animals respond differently to equivalent doses of a chemical substance than younger animals; and (b) are there differences in response between various strains of a species? Heroux *et al.* (3) reported that the optimal dose of tyramine to elicit a maximum metabolic response in young male Sprague–Dawley (S–D) rats was 20 mg/kg body weight intraperitoneally. It is of practical interest to determine whether this optimal dose of tyramine for young animals is optimal for older (2 years old) animals. Available data revealed that male Sprague–Dawley rats have a median survival rate of 23 (12), 24.5 months (13, 14), and 30 months (15), while male Fischer 344 (F344) rats have a median survival rate of 27.5 (16) to 29 months (17). There are significant differences in mean body weights between the two strains of male rats. At 80 to 90 days of age, male Sprague–Dawley had a mean weight of 325 g while F344 weighed 214 g (18). At 20 to 24 months, male Sprague–Dawley attained mean body weights of 700 g (13) while male F344 had a mean weight of 329 g (19).

There is minimal information differentiating the responses of these strains to a stimulus, either chemical or physical. Such knowledge is imperative in selecting the appropriate strain of animal for experimental use. This paper presents the differences in metabolic response to tyramine in relation to age; i.e., young and old, and between the two strains of rat commonly utilized for aging research, Sprague–Dawley and Fischer 344.

Materials and Methods. Male Sprague–Dawley and Fischer 344 rats, 3 to 4 and 20 to 24 months of age, were studied. The animals were individually caged and provided with Purina Laboratory Chow and water *ad libitum*. A 12-hr light and dark cycle was maintained. Oxygen uptake was

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continuously measured in a constant volume, closed system (Volume Meter, Med Science, Model 160) with the rat in a sealed Plexiglas chamber while immersed in a 28° water bath, and the expired CO_2 was absorbed by soda lime. Colonic and chamber temperature were measured continuously with thermistor probes and recorded on a dual-channel recorder. Before evaluating the animals' response to tyramine, O_2 uptake and colonic temperature were first measured at 28° for 30 min to establish a baseline. Each animal was then temporarily removed from the chamber and given intraperitoneal tyramine–HCl. The animal was then returned to the chamber and measurements resumed for another hour. The resulting data were plotted and the peak response to tyramine which was generally present for several minutes was calculated. At least one week elapsed between each administration of tyramine in the same animal.

The optimal dose of tyramine–HCl administered was determined by constructing a log-dose–response (LDR) curve for each of the four groups of rats. That is, successively increasing log doses of tyramine–HCl were administered to the rats, and the metabolic response was plotted against log dose until the response curve plateaued when a higher dose of tyramine–HCl was administered. The optimal dose was defined as the lowest dose of tyramine–HCl that produced a maximal response increase in O_2 uptake and colonic temperature. The significance of the differences between O_2 uptake and colonic temperature before and after administration of tyramine was determined by using paired *t* tests.

Results. The increases in O_2 uptake and colonic temperature following tyramine injections were statistically significant from each basal value in all rats and at all dosages tested ($p < 0.001$), except in the old Sprague–Dawley rats when tested with the 20 mg/kg dose. The optimal dose (20 mg/kg bw) for young Sprague–Dawley failed to elicit a significant increase in either O_2 uptake or colonic temperature in old Sprague–Dawley rats.

Young rats. Young rats respond to an injection of tyramine with increases in O_2 uptake and colonic temperature. O_2 uptake usually peaks within 10 to 20 min following the injection and then gradually subsides and returns to basal levels in an hour or so. Peak response increases with increasing dosages until an optimal dose is reached and a plateau in response occurs. Elevation of the colonic temperature follows the rise of O_2 uptake with a time lag of 5 to 10 min. The degree of elevation corresponds with increasing dosage and then plateaus when the optimal dose is reached.

In young Sprague–Dawley ($n = 9$), three dosages (5, 10, and 20 mg/kg body weight) were used and 20 mg/kg body weight was determined to be the optimal dose (Figs. 1 and 2). A higher basal metabolic rate was observed in the young Sprague–Dawley given 20 mg compared to those given 10 mg. This change was probably related to the 2-week interval between test sessions when these young rats grew rapidly and effect of age on metabolism became noticeable. Since in some rats this dose elevated colonic temperature to 40.6°, any additional rise of colonic temperature was considered to be potentially harmful, and since in additional studies on cold-acclimated animals this tyramine dose resulted in colonic temperature approaching 42°, no higher dosage than 20 mg/kg body weight was attempted in this group.

Young F344 ($n = 13$) were initially tested with 5, 10, and 20 mg/kg body weight dosages. No obvious differences in their response could be discerned. Therefore, another group of young F344 ($n = 13$) were reevaluated at lower dosage levels (0.5, 1, and 2 mg/kg body weight). The optimal dose for young F344 was thus determined to be 2 mg/kg body weight (Figs. 1 and 2).

Old rats. Basal O_2 uptakes are lower in old animals, approximately 16 to 19 $ml \cdot kg^{-1} \cdot min^{-1}$, as compared to 21 to 27 $ml \cdot kg^{-1} \cdot min^{-1}$ in young animals. The old Fischer rats have higher basal colonic temperature (approximately 0.8° higher) than young Fischer. However, old animals also respond to tyramine by increases in O_2 uptake and colonic temperature. Most signifi-

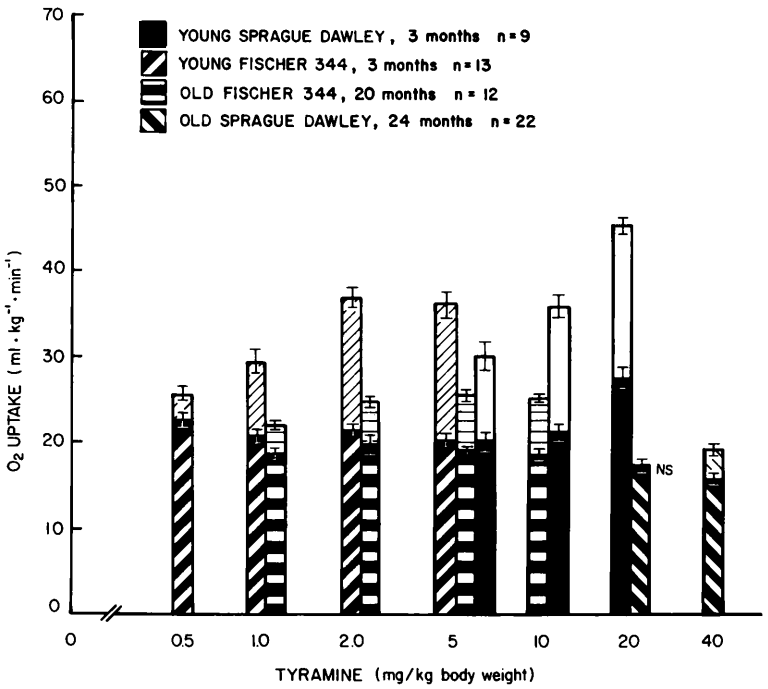

FIG. 1. Increases in oxygen uptake (clear portions of the histograms) in response to intraperitoneal injections of tyramine. All measurements were made in an ambient environment of 28°. Error bars represent SEM.

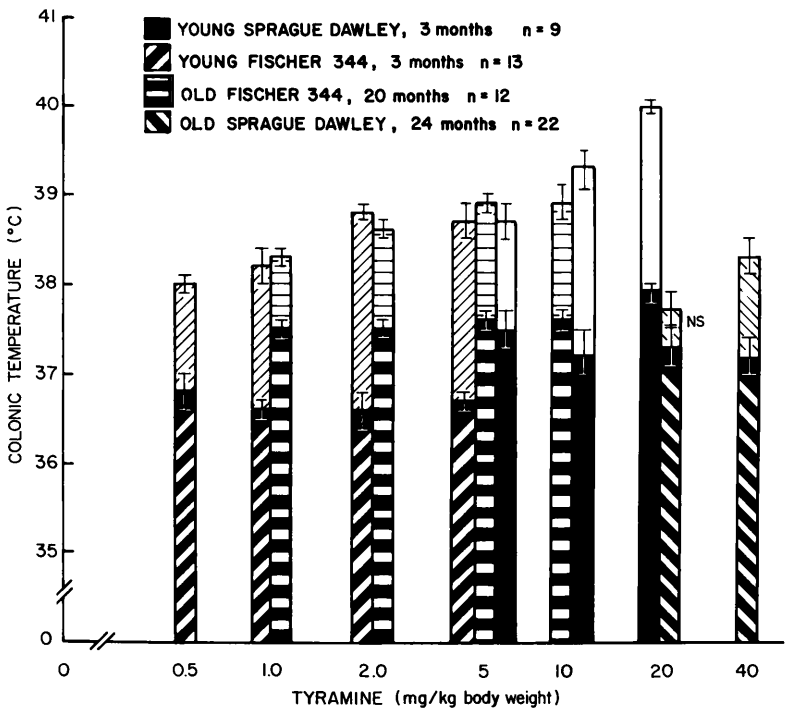

FIG. 2. Elevation of colonic temperature in response to intraperitoneal injections of tyramine. All measurements were made in an ambient environment of 28°. Error bars represent SEM.

cantly, the magnitude and time course of the response differs strikingly from the responses of the younger animals. The elevations of O_2 uptake and colonic temperature are smaller. The time course of change of O_2 uptake is similar to that of the young, but the time course for the change of colonic temperature is altered. Peaking of colonic temperatures is usually delayed 50 to 60 min. Two hours or more are required before basal levels are reattained.

The optimal dose for old F344 ($n = 12$) was determined to be 5 mg/kg body weight. Although the increase of O_2 uptake was similar at 2, 5, and 10 mg/kg doses, the elevation of colonic temperature apparently peaked at 5 mg and plateaued at 10 mg (Figs. 1 and 2). The optimal dose (20 mg/kg body weight) for young Sprague–Dawley did not elicit any significant response in old Sprague–Dawley ($n = 5$) (Figs. 1 and 2). A dose of 40 mg/kg body weight was then given and significant increases in O_2 uptake and colonic temperature ($P < 0.001, n = 22$) were obtained. No higher dosage was attempted at this point; the optimal dose for old Sprague–Dawley being apparently greater than 40 mg/kg body weight.

Discussion. The optimal doses of tyramine for each group of rats studied (2 mg/kg body weight for young F344, 5 mg/kg body weight for old F344, 20 mg/kg body weight for young Sprague–Dawley, and >40 mg/kg body weight for old Sprague–Dawley) represent a significant difference between young and old in each respective strain and between strains of either young or old animals.

The sluggish and prolonged response in old animals may be explained by altered absorption, metabolism, or excretion of drugs in old animals (20, 21). It is known that the myocardial function and blood flow to organs and tissues in old animals are reduced (22). There are functional alterations in liver and kidney (22). However, the high optimal doses found for old animals cannot be explained by these altered mechanisms alone, since old animals had been shown to be more sensitive to drugs, especially to norepinephrine (8), than young ones (21). Since tyramine acts by release of endogenous NE, this increased rather than decreased dose requirement in older animals could be due to:

(1) Decreased NE synthesis in old animals. Aging impairs the uptake of tyrosine or dopa into neurons as well as the intracellular hydroxylation of tyrosine and the β-oxidation of dopamine (7). Therefore, the available NE for release by tyramine is smaller, and more tyramine is required to produce an apparent effect. Diminished availability of NE can also be responsible for the reduced metabolic effect observed in old animals.

(2) Decreased re-uptake of NE in nerve endings in aging animals. Since tyramine is taken up at the nerve ending by the same amine-pump mechanism, the uptake of tyramine can also be slowed (7). Reduced re-uptake of NE indicates a reduced availability of NE for release by tyramine.

(3) Increased monoamine oxidase (MAO) activity in old rats. Both NE and tyramine are substrates for MAO. Increased MAO activity may result in reduced available NE and tyramine (23).

The prolonged response or decreased rate of recovery in old animals represents an example of the often observed phenomenon that the aged require more time than the young to reestablish homeostasis when displacements are induced (22). It is also of interest that we found a relatively lower basal O_2 uptake but higher basal colonic temperature in old F344 than in young F344. This may suggest more efficient heat production, less effective heat dissipation, or better insulation in older F344.

The strain-related difference observed in this study is of considerable significance since Sprague–Dawley and F344 are the two most commonly used strains in age-related studies. Mazze *et al.* (24) compared the rate of metabolism of methoxyflurane to inorganic fluoride and in its nephrotoxic effects among five rat strains: Fischer 344, Buffalo, Wistar, Sprague–Dawley, and Long–Evans. F344 rats were found to be more susceptible to inorganic fluoride than all other strains. They suggested that genetic factors were responsible for the difference. Although some other unknown fac-

tors may be responsible for the difference in response between the F344 and Sprague–Dawley rats observed in this study, we are inclined to accept the suggestion that genetic factors were responsible for the difference.

From our data it is apparent that generalizations as to the physiological alterations induced or present in aging animals cannot be made lightly. Whether or not the strain differences observed have a direct effect on aging processes remains to be determined.

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