

Tissue Isotope Clearance

III. Variability Attributed to Age, Sex, and Core Temperature in Rats (38317)

LOUIS P. GANGAROSA, CHING T. HUNG, AND P. KENNETH MORSE

Department of Dental Pharmacology, Medical College of Georgia, Augusta, Georgia 30902

While conducting experiments to determine drug effects upon subcutaneous isotope clearance using the method of Kety (1), it was observed that control clearances varied appreciably in successive groups of rats. Some of these groups were known to differ in sex and age. Also, animals anesthetized with a barbiturate (which was used in these experiments) tend to have depressed core temperatures which may vary with the ambient temperature. It was hypothesized, therefore, that differences in age, sex and core temperature might account for the observed variability in subcutaneous isotope clearance. Two studies (2, 3) have shown that isotope clearance rates from injection sites can vary with core temperature. However, no specific studies demonstrating the effects of age and sex and their interaction with core temperature on isotope clearance rates have been found.

The purpose of the present study was to test the effects (both independent and interactive) of age, sex and core temperature upon isotope clearances following injection of the isotope in saline solution.

In addition, two local anesthetics, prilocaine and tetracaine which were reported to have opposite effects upon clearance rate (4) were added to the isotope solutions as positive controls under all the conditions of the experimental design.

Methods. All rats used were of the Strain CD-1 (Sprague-Dawley derived) and were obtained from Charles River. Nine rats of each sex in each of four designated age categories were selected and randomly assigned to three core temperature ranges, 93.5°-95.5°, 100°-102°, and 105.0°-107°F.

The age categories were: (a) immature (about 45 days old), (b) pubescent (about 84 days old), (c) mature (about 5 mo old) and (d) aged (about 12 mo old). Each rat was anesthetized with a

mixture of pentobarbital (35 mg/kg) and barbital (90 mg/kg) and was equilibrated to one of the three core temperature ranges by adding or removing radiant heat sources. Core temperature was monitored with a Yellow Springs Rectal thermister and a tele-thermometer, YSI Model 47. Three subcutaneous injections were given in random sequence to each rat at nearby sites in the dorsal-lumbosacral midline. Each injection solution contained 0.1 ml of ¹³¹I-4-iodoantipyrine (IAP) in saline-phosphate buffer (pH7.4) at physiologic osmolality. In addition, the positive control injections contained either 0.1% tetracaine or 2.0% prilocaine with appropriate adjustment of osmolality and pH. The clearance of IAP from the injection site was monitored with a Nuclear Chicago surgical scintillation probe (Model 931) which was connected to an appropriate recording apparatus. Twenty min were allowed between injections for clearance determinations. All drugs were mixed freshly from powders on the day of the experiment. Detailed methods were described previously (4).

The data were analyzed separately for each drug condition as a 4 × 3 × 2 completely crossed, fixed effects analysis of variance design. During the experiments, two aged rats died; the grand mean was substituted for the clearance values for the appropriate drug and animal variable and the degrees of freedom were reduced by two. This is a conservative procedure and tends to reduce significant differences.

Results. The results of the three analyses are summarized in Table I. When the IAP injection was given in saline, significant independent effects were obtained for sex, body temperature, and the interaction between age and sex. The source of variability having the most significant effect under this condition was sex. Clearances

TABLE I. Summary of the Effect of Age, Sex, and Temperature Upon Subcutaneous Tissue Isotope Clearance Under Three Drug Conditions^a.

Variables	Drug conditions		
	No Drug (Saline Buffer)	2% Prilocaine	0.1% Tetracaine
Age	NS	$P < 0.001$	$P < 0.001$
Sex	$P < 0.001$	NS	$P < 0.01$
Core Temp	$P < 0.05$	$P < 0.001$	$P < 0.01$
Age $\times$ Sex	$P < 0.05$	NS	$P < 0.01$
Age $\times$ Temp	NS	NS	NS
Sex $\times$ Temp	NS	NS	NS
Age $\times$ Sex $\times$ Temp	NS	NS	NS

^a NS = not significant at 0.05 level.

obtained from an injection of an IAP solution containing 2% prilocaine, a depressant of clearance (4), identified age and temperature as significant sources of variation, while the main effect for sex and the age-sex interaction noted for nondrug clearance were no longer apparent. When using the IAP solution with 0.1% tetracaine, which has a stimulant effect on clearance (4), the significant sources of variability were age, sex and temperature, as well as, the age-sex interaction.

By plotting temperature against the mean initial clearance rate, the temperature effect can be visualized under the different drug conditions (Fig. 1). There was an increasing clearance with increased core temperature irrespective of whether or not a drug was added to the solution.

Figure 2 shows graphically the significant age-sex interactions in the presence of no drug and in the presence of tetracaine. The greatest differences between the sexes occur at ages II and III (refers to "no drug" curves) with the males having the higher clearance rate at these ages.

In both Figs. 1 and 2 the difference between the drugs is clearly shown, tetracaine (0.1%) having a higher clearance rate than "no drug" which was higher than prilocaine (2%).

Discussion. These experiments show that differences in clearance rates of injected isotopes are related to animal age, sex and core temperature. The effects of animal variables upon clearance rate have not previously been studied except for hypothermia. Westin *et al.* (2, 3) concluded that clearance rates of radiosodium or radiobromide were correlated directly with degree of hypothermia. Our results with IAP clearances agree with the latter authors. As far as can

be determined, we are the first to report the increased isotope clearance rate during hyperthermia, although McGirr (5) reported effects of local and reflex heating and cooling on $^{24}\text{Na}^+$ clearance.

In a previous report (4), we showed that local anesthetics generally depressed local blood flow as evidenced by inhibited isotope clearance when the local anesthetic was added to the isotope solution and injected into subcutaneous tissue. This depression was concentration related for all anesthetics tested. A notable exception was tetracaine, which at a low concentration (0.5%), stimulated blood flow. In the present study, we verified the previous observations.

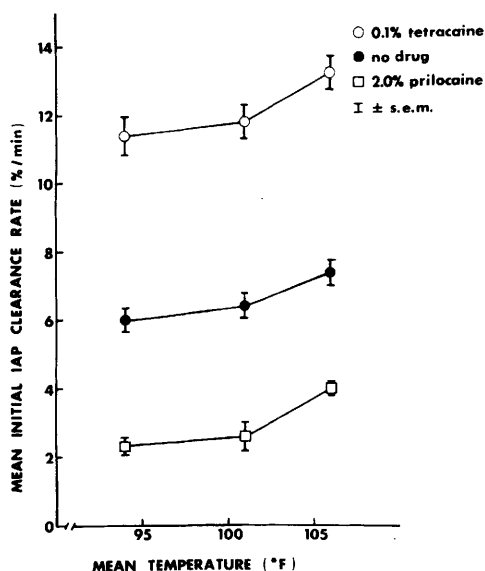


FIG. 1. Mean clearance values for three temperature levels under three drug conditions.

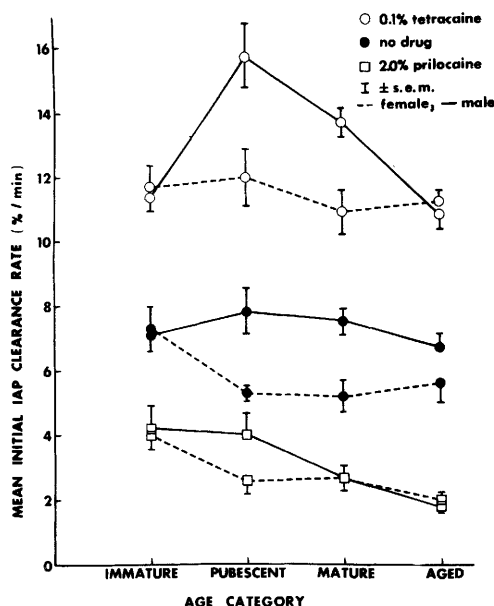


FIG. 2. Mean clearance values for age $\times$ sex interaction under three drug conditions.

These local anesthetic effects upon clearance were noted in both sexes and all four age groups (Fig. 2) and for all three core temperatures (Fig. 1).

Animal maturation and aging effects upon clearance were significant sources of variation in the presence of tetracaine or prilocaine, but not under the condition of no drug. Generally there was decreased clearance in Age IV under all three conditions (Fig. 2) which is compatible with the general depression of blood flow which accompanies the aging process. However, the effects seen from Ages I to II and from II to III are more complex, apparently involving sexual maturation. The males in the sample generally had greater clearance rates than females especially at Ages II and III (Fig. 2). This significant age $\times$ sex interaction was observed with either no drug or tetracaine, and it suggests that hormones are influencing subcutaneous blood flow. Hormonal effects upon blood flow have been shown for target organs, such as uterus, which shows an increased vascularity and flow related

to cyclical changes (6). Although there is a tendency for female sex organs to increase in blood flow under influence of hormones, there may be compensatory decreases in other organs (such as skin). Alternatively, the estrogens may have direct effects upon other vascular beds and such effects are likely to be opposed by androgens.

It was of interest also that the vasoconstrictor local anesthetic, prilocaine, had a more pronounced effect in aged rats, suggesting that the mechanism causing inhibition of isotope clearance by local anesthetics is accentuated by aging. Luduena (7) reviewed the mechanism of this depressant effect on blood flow. It is thought to be due to a mild vasoconstrictive effect of prilocaine attributed mostly to its levorotary isomer.

These results indicate that research studies using clearance rates as a dependent variable should specify the age, sex, and core temperatures of the subjects. While it is unlikely that such animal variables would mask the difference in clearance between a pronounced stimulant of clearance (e.g., tetracaine) and a pronounced depressant of clearance (e.g., prilocaine), they could easily distort findings for two drugs with similar effects or where the differences between the drugs are not so clear cut.

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