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Effects of Phenobarbital on Cerebral Blood Flow and Metabolism in Young and Aged Rats

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The cerebrovascular and cerebral metabolic changes produced by intraperitoneal injection of phenobarbital (50, 150, and 250 mg/kg) were studied in young adult (6-month) and senescent (28-month) Wistar rats. Cerebral blood flow (CBF) was measured using radioactive microspheres and cerebral oxygen consumption (CMR_{O_2}) was obtained by multiplying cortex CBF by the arterial–sagittal sinus oxygen content difference. Control values for blood pressure, blood gas tensions, CBF, and CMR_{O_2} were similar in the young and aged animals during 70% N_2O/30% O_2. Intraperitoneal phenobarbital produced dose-dependent decreases in CBF with no significant difference between young and aged rats at each phenobarbital dose. At the highest phenobarbital dose (250 mg/kg) CBF was reduced by 49% in the young rats and 52% in the aged rats ($P > 0.10$). CMR_{O_2} was also depressed in a dose-dependent fashion in both young and aged animals with each phenobarbital dose. However, the decrease produced by the highest phenobarbital dose was significantly greater in the aged rats (55%) than the young rats (43%, $P < 0.05$), even though the EEG was isoelectric in both groups. The difference in CMR_{O_2} between young *versus* aged rats at a time when the EEG is isoelectric suggests that high-dose phenobarbital may depress nonelectrical cerebral metabolic processes more in aged rats. (Key words: Age factors. Anesthetics, intravenous: phenobarbital. Brain: blood flow; metabolism.)

IT IS WELL established that decreases in cerebral blood flow (CBF) and cerebral oxygen consumption (CMR_{O_2}) produced by barbiturates correlate closely with the degree of anesthesia and EEG suppression.[1] CBF and CMR_{O_2} are maximally depressed with barbiturate doses that produce a quiescent EEG. Nonelectrical cerebral metabolic activity is resistant to further depression with higher barbiturate doses.[1,2] Little is known about how the cerebrovascular and cerebral metabolic responses induced by barbiturate anesthesia may be altered with aging. Young and aged rats were tested using a model that has previously demonstrated phenobarbital-induced decreases in CBF, CMR_{O_2}, and EEG activity.[2]

Methods

Thirty-seven young (6-month) and 32 aged (28-month) Wistar rats were anesthetized with halothane and, following tracheostomy, were artificially ventilated with 1% inspired halothane in 70% nitrous oxide/30% oxygen using a small animal respirator. Catheters were inserted into both femoral arteries and a femoral vein for fluid and drug administration, blood sampling, and hemodynamic monitoring. A catheter for microsphere injections was inserted into the left ventricle *via* the right carotid artery. Proper placement of the left ventricular catheter was assured by observing a change in the pressure tracing from an arterial to a left ventricular pattern. The animal was then placed in the prone position, the skull was exposed, and a small burr hole was drilled over the posterior sagittal sinus. A needle-tipped catheter was fixed into the hole for withdrawal of blood samples used to determine cortical venous oxygen content. Stainless steel screw electrodes were applied bilaterally over the parietal cortices for continuous bipolar EEG monitoring. A third screw electrode over the frontal cortex served as a ground electrode. All leads were shielded to prevent 60-cycle interference. The EEG was recorded using a Grass Instruments® P15 differential AC amplifier and a Hewlett-Packard® strip chart recorder. Filter settings were 1 Hz at the low range and 30 Hz at the high range.

Following surgical preparation halothane was discontinued and the rat was immobilized with 1 mg/kg tubocurare and allowed to stabilize for 45 min while being ventilated with 70% N_2O/30% O_2. Arterial P_{CO_2} was adjusted to 35–40 mmHg and arterial P_{O_2} was maintained greater than 100 mmHg. Rectal temperature was measured with a thermistor probe and maintained at 37° C using an overhead heat lamp. Mean arterial blood pressure

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was recorded continuously from a femoral arterial catheter. Heart rate was measured from arterial pressure pulses periodically throughout the experiment.

At the end of the stabilization period each rat received an intraperitoneal injection of one of three phenobarbital doses (50, 150, or 250 mg/kg) or sham treatment (normal saline). CBF was measured 90 min later using radioactive microspheres. Arterial and sagittal sinus blood samples (0.2 ml each) were taken immediately after the microsphere test for measurement of blood gas tension and *p*H using an IL 1303 (Instrument Laboratories, Lexington, MA) blood gas analyzer and for determination of arterial and sagittal sinus (venous) oxygen content using an IL 282 co-oximeter. Sagittal sinus samples were drawn over 10–15 s. Because the sagittal sinus drains primarily cerebral cortex, these venous samples reflect cortical oxygen extraction. Cortical CMR_{O_2} was calculated by multiplying cortical blood flow by the arterial–sagittal sinus oxygen content difference (AV_{O_2}).[2] EEG was recorded throughout the 90-min phenobarbital treatment period. Preliminary studies indicated that the maximum effect of ip phenobarbital was produced by 90 min, with little to no additional EEG suppression thereafter.

To measure CBF, 15-μm microspheres labeled with cobalt-57 (New England Nuclear, Boston, MA) were suspended in a stock solution of isotonic saline with 0.01% Tween-80®. After the solution was vortexed, 0.2 ml (100,000 microspheres) was injected into the left ventricular catheter and flushed in with 0.2 ml isotonic saline over 20 s. Starting immediately before the microsphere injection, blood was withdrawn from a femoral artery catheter at a rate of 0.4 ml/min using a Harvard infusion/withdrawal pump. A withdrawal period of 45 s was chosen to ensure the removal of all circulating microspheres. At the end of each microsphere test arterial and sagittal sinus blood samples for blood gas tension, *p*H, and oxygen content were obtained. Blood pressure was continuously monitored throughout the microsphere testing to ensure no hemodynamic changes prior to CMR_{O_2} determination. The rat was then killed, and the brain was removed, divided into left and right cortical and subcortical samples, weighed, and placed in counting tubes for analysis of radioactivity. The activity of the microsphere label was analyzed in blood and brain tissues with a Nuclear Chicago® 600 multichannel analyzer. CBF was calculated as follows:

$$\text{CBF}(\text{ml}/100\,\text{g}/\text{min}) = \frac{\text{Tissue activity}}{\text{Blood sample activity}} \times \frac{\text{Blood sample withdrawal rate}}{\text{Tissue weight}} \times 100$$

Cerebrovascular resistance was calculated from CBF and mean blood pressure measured at the time of microsphere injection.

When the higher phenobarbital doses produced hypotension, methoxamine was infused intravenously to maintain mean arterial blood pressure higher than 100 mmHg. Separate experiments were first carried out to ensure that methoxamine had no direct cerebral vasoconstrictor effect. Cortical and subcortical blood flow was measured in four rats during 70% N_2O/30% O_2 using cobalt-57–labeled microspheres and then during a methoxamine infusion that raised mean blood pressure approximately 25 mmHg using a second microsphere labeled with tin-113.

Data are reported as mean ± SE. The effect of phenobarbital anesthesia on young and aged rats was compared using a two-way analysis of variance. Multiple tests comparing the means of young and aged rats at each phenobarbital dose were performed using unpaired *t* tests with a Bonferroni correction. This correction reflected the four tests of pairs for each parameter measured.

Results

In preliminary validation studies, methoxamine infusion increased mean blood pressure from 133 ± 4 to 158 ± 6 mmHg (mean ± SE; n = 4) with no significant change in CBF. Cortical and subcortical blood flows were 125 ± 16 and 69 ± 5 ml/100 g/min, respectively, in control state and 125 ± 7 and 71 ± 5 ml/100 g/min, respectively, during the methoxamine infusion. Based on these data we concluded that methoxamine could be used to maintain blood pressure during phenobarbital treatment with little effect on cerebral resistance vessels.

The effects of phenobarbital on blood pressure, arterial blood gases, and *p*H are shown in table 1. Arterial blood pressure of young and aged rats was similar during nitrous oxide control conditions and decreased in both groups with increasing phenobarbital doses. A methoxamine infusion was necessary to maintain a mean arterial pressure greater than 100 mmHg in all young and aged rats treated with 150 mg/kg and 250 mg/kg phenobarbital. Pa_{CO_2} was maintained between 35 and 40 mmHg with mechanical ventilation, and Pa_{O_2} remained higher than 100 mmHg in all treatment groups. There were no significant intergroup differences in blood pressure and blood gas tensions. Arterial *p*H and P_{O_2} increased in both young and aged rats following treatment with phenobarbital.

The effects of phenobarbital on EEG are shown in figure 1. EEG changed from low-amplitude, high-frequency activity during nitrous oxide to high-amplitude, low-frequency waves with 50 mg/kg phenobarbital. This activity was further depressed with 150 mg/kg phenobarbital,

TABLE 1. Arterial Blood Pressure, Blood Gases, and *p*H in Phenobarbital-anesthetized Young and Aged Rats

Phenobarbital (mg/kg)	n	Blood Pressure (mmHg)	Pa_{CO_2} (mmHg)	Pa_{O_2} (mmHg)	*p*H
Young					
0	9	138 ± 6	37.7 ± 1.2	121 ± 9	7.37 ± .02
50	10	126 ± 1	37.8 ± 0.3	155 ± 2	7.44 ± .01*
150	9	122 ± 2*	35.2 ± 0.1	173 ± 2*	7.43 ± .01
250	9	115 ± 1*	36.0 ± 0.2	159 ± 6*	7.41 ± .01
Aged					
0	7	137 ± 4	35.8 ± 0.8	116 ± 7	7.39 ± .01
50	8	123 ± 6	40.2 ± 0.9	148 ± 18	7.45 ± .01*
150	9	127 ± 3	36.9 ± 1.0	181 ± 13*	7.45 ± .01*
250	8	118 ± 4*	35.8 ± 0.9	181 ± 9*	7.41 ± .01

There were no intergroup differences in any of the parameters measured.

* $P < 0.05$ compared with zero dose control for each age group.

and the EEG was isoelectric with the 250 mg/kg dose in both age groups. Little difference between young and aged rats was seen in the development of EEG changes over the 90-min phenobarbital treatment period or in the EEG records at the time of testing.

CBF changes produced by phenobarbital paralleled the changes in EEG (fig. 2). Control CBF values were similar in young and aged rats. Phenobarbital produced a dose-dependent decrease in cortical and subcortical CBF in both age groups. This depression was significant in both young and aged rats as compared with nitrous oxide control values. The maximal decrease in cortical blood flow was approximately 50%, whereas the maximal depression of subcortical flow was 33%, with no difference in response between the two age groups ($P > 0.10$). Cerebrovascular resistance (CVR) was also similar between young and aged rats. Cortical CVR increased from 1.18 ± 0.10 $mmHg \cdot ml^{-1} \cdot 100\ g^{-1} \cdot min^{-1}$ under control conditions to a maximum of 2.08 ± 0.07 $mmHg \cdot ml^{-1} \cdot 100\ g^{-1} \cdot min^{-1}$ at the highest phenobarbital dose in young rats. In aged rats, cortical CVR increased from 1.15 ± 0.16 to 2.00 ± 0.10 $mmHg \cdot ml^{-1} \cdot 100\ g^{-1} \cdot min^{-1}$. Analyses of variance indicated that cortical and subcortical CVR increased significantly during phenobarbital treatment ($P < 0.01$) and that these increases were not significantly different between age groups ($P > 0.10$).

Cerebral metabolism was also depressed in both young and aged rats following phenobarbital administration (fig. 3). As with CBF, phenobarbital-induced metabolic depression reached a plateau in both young and aged rats at the 150–250 mg/kg doses. Analysis of variance indicated that significant decreases in CMR_{O_2} were produced with phenobarbital in both age groups ($P < 0.01$), and this depression was greater in aged (55%) as compared with young rats (43%; $P < 0.01$). A difference in response to phenobarbital in young *versus* aged rats was also suggested by changes in AV_{O_2}. AV_{O_2} was not significantly different between young and aged rats during 70% N_2O or following 50 mg/kg phenobarbital (70% N_2O, young = 5.8 ± 0.4 ml O_2/100 ml, and aged = 6.0 ± 0.9 ml O_2/100 ml [$P > 0.10$]; 50 mg/kg phenobarbital, young = 5.9 ± 0.1 ml O_2/100 ml, and aged = 5.9 ± 0.6 ml O_2/100

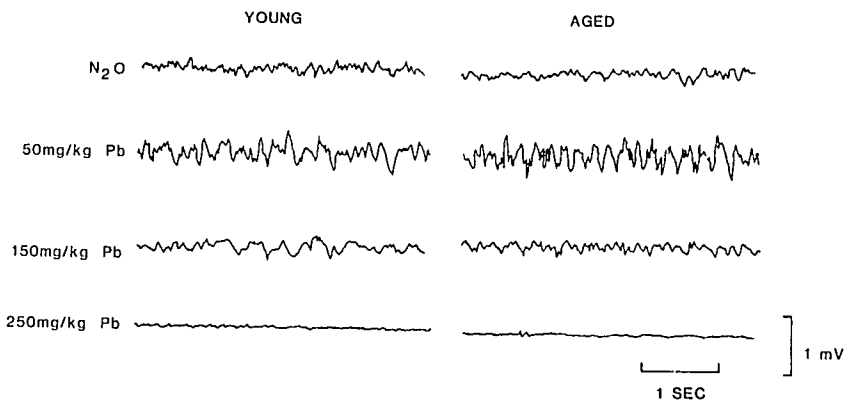

FIG. 1. EEG changes during phenobarbital (Pb) treatment in young and aged rats (mV = millivolts). Each EEG strip represents a separate rat and treatment condition. Phenobarbital produced dose-related depression of EEG activity in both young and aged rats and complete suppression of electrical activity at the 250 mg/kg dose. The time sequence of EEG changes, not shown here, was also similar between young and aged rats.

ml [$P > 0.10$]). Cerebral oxygen extraction increased in young compared with aged rats with 150 and 250 mg/kg phenobarbital (150 mg/kg, young = 7.3 ± 0.1 ml O_2/100 ml, and aged = 5.7 ± 0.3 ml O_2/100 ml [$P < 0.05$]; 250 mg/kg, young = 6.8 ± 0.1 ml O_2/100 ml, and aged = 5.5 ± 0.3 ml O_2/100 ml [$P < 0.05$]).

Discussion

Twenty-eight-month-old rats have been physiologically characterized as senescent in previous studies, approximately equivalent to a 70-yr-old human when the life spans of rats and humans are equated.[3-5] The 6-month-old rats used in this study may be considered as a young adult.[3] In these studies, ip phenobarbital produced similar dose- and time-related EEG suppression that reached a maximum in 90 min in young and aged rats. This suggests that brain uptake of the barbiturate was similar between the two age groups over the 90-min interval. This agrees with the report of Kapetanovic *et al.*[6] that plasma and brain phenobarbital concentrations peak at approximately 90 min and are similar in young and aged rats up to 2 h after ip injection. CBF was decreased in a similar manner in young and aged rats following phenobarbital, but CMR_{O_2} decreased more in aged rats with higher phenobarbital doses. Because EEG was isoelectric at the highest phenobarbital dose, a difference in neuronal electrical activity between the two age groups is not likely.

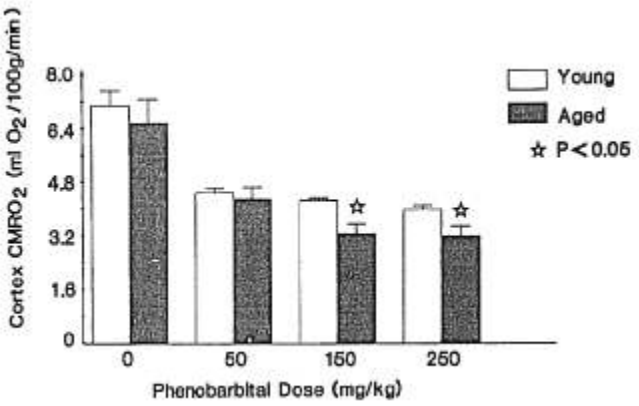

FIG. 3. Cortical CMR_{O_2} changes during phenobarbital anesthesia in young and aged rats. Significant differences between young and aged, as determined by *t* test and indicated by ☆, are shown at 150 and 250 mg/kg phenobarbital doses. Analysis of variance indicates a significant decrease in CMR_{O_2} in both young and aged rats as compared with control with increasing phenobarbital doses ($P < 0.01$), with a greater decrease in the aged *vs.* young rats ($P < 0.01$).

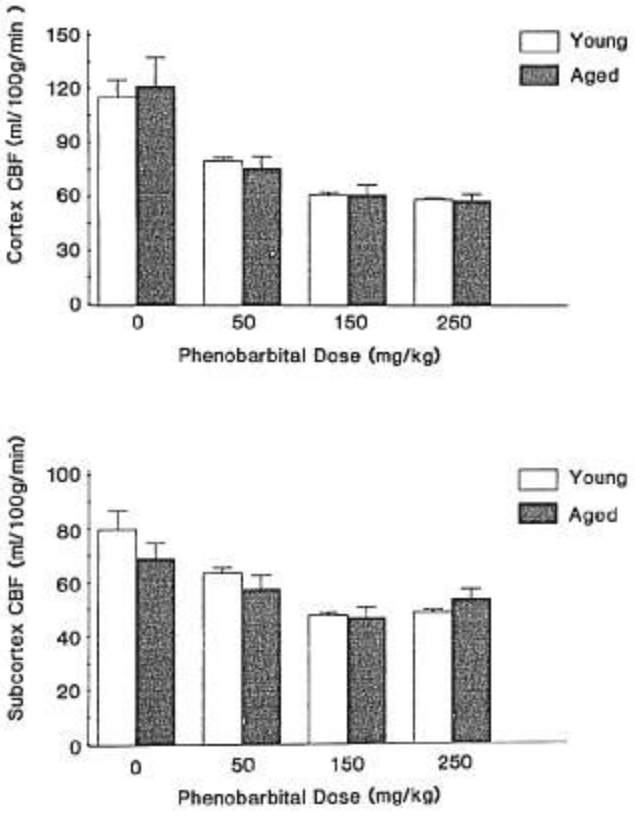

FIG. 2. Cortical and subcortical blood flow in young and aged phenobarbital-anesthetized rats. Analysis of variance indicates significant blood flow depression with increasing phenobarbital doses ($P < 0.01$) in both young and old rats as compared with control. There was no significant difference in CBF between young and aged rats at control or any phenobarbital dose ($P > 0.10$).

Nitrous oxide was used as the control anesthetic because it provides analgesia in rats with no depression or stimulation of CBF or brain metabolism.[7,8] Although the work of Seyde and Longnecker[9] suggests that CBF in unanesthetized rats may be lower than values reported here with N_2O, others have reported that cortical CBF in unanesthetized rats is not different from N_2O-ventilated animals and is similar to our values.[7] N_2O apparently produces little stress in rats because CBF and CMR_{O_2} are not elevated compared with unstimulated adrenalectomized rats or rats pretreated with beta blockers.[10] Withdrawal of N_2O from paralyzed rats results in stress-related increases in both CBF and CMR_{O_2}.[10] It is also possible that N_2O may alter the cerebrovascular or metabolic response to phenobarbital. In a recent report, Sakabe *et al.*[11] found in rats that N_2O attenuates the decrease in cerebral glucose consumption produced by pentobarbital (30 mg/kg), but it does not alter cerebral metabolic depression or EEG flattening produced by 125 mg/kg pentobarbital. This suggests that N_2O may produce a small stimulation of CMR_{O_2} in rats during low-dose barbiturate anesthesia;

however, N_2O probably has little effect on cerebral metabolism and EEG depression produced by high-dose phenobarbital.

Both young and aged rats showed dose-related decreases in CMR_{O_2} that were not different under control conditions and with 50 mg/kg phenobarbital. These results were consistent with EEG changes and suggest that sensitivity to phenobarbital was similar between the two age groups. This agrees with reports that the brain sensitivity to thiopental does not change with age.[12] The aged rats, however, showed more cerebral metabolic depression than young rats at higher phenobarbital doses. Because electrical neuronal activity is absent with the highest phenobarbital dose in both age groups, the lower CMR_{O_2} observed in aged rats may represent a decrease in metabolic activity devoted to nonelectrical neuronal function. This suggests that elderly patients may be more sensitive to barbiturates, based not only on pharmacokinetic differences such as decreased drug clearance, changes in body composition, and drug volume of distribution,[12,13] but that there may also be a pharmacodynamic effect of greater cerebral metabolic depression at high doses.

There are two ways to interpret the cerebrovascular effects of phenobarbital observed in this study. The first is to analyze the direct cerebrovasoconstrictor effects associated with increasing doses of the drug. By this analysis, phenobarbital decreased CBF and increased cerebrovascular resistance to the same extent in both young and aged rats. The second way is to relate decreases in CBF during phenobarbital treatment to simultaneous decreases in CMR_{O_2}. Since the early report of Roy and Sherrington[14] it has been generally observed that both regional and global changes in CBF are tightly coupled to changes in brain metabolism. However, it has been observed previously[2] as well as in this study that CBF decreases more than CMR_{O_2} in young phenobarbital-treated rats, producing an increase in cerebral oxygen extraction. This may be interpreted as an additional cerebrovasoconstrictor effect, which is greater than that required by the decrease in brain metabolism.

There are two possible mechanisms by which phenobarbital could mediate the additional cerebrovasoconstriction that is seen in young but not aged rats. The first is by a direct vasoconstrictor effect of the drug on cerebral resistance vessels, which has been suggested previously.[15] This appears unlikely since *in vitro* experiments on rat aorta strips by Altura and Altura[16] and on isolated cerebral vessels by Marin *et al.*[17] and Edvinsson and McCulloch[18] have shown that barbiturates produce vascular smooth muscle relaxation, not constriction. Recently, however, Fukuda *et al.*[19] reported that thiopental potentiated alpha-adenoreceptor–induced vasoconstriction in artery smooth muscle preparations. Similar vascular adenoreceptors have been identified in human and rat cerebral vessels.[20] It is also possible that the *in vivo* effects of phenobarbital do not correspond to *in vitro* actions.[21]

The second possible mechanism by which barbiturates may produce vasoconstriction is by decreasing the sensitivity of vascular smooth-muscle receptors to endogenous mediators of local vasodilation, such as potassium, H^+, or adenosine.[22] Such an effect would decrease local cerebral perfusion and increase oxygen extraction requirements for the perfused tissue, as was observed in young rats. If phenobarbital produces cerebrovasoconstriction by one of the earlier mentioned mechanisms, a lack of this effect in aged rats may be due to a change in sensitivity to this phenobarbital-induced vascular action.[23,24] This may occur independently from the direct cerebral metabolic depressant effects of phenobarbital in young *versus* old animals.

In summary, while both young and aged rats show dose-related decreases in CBF and CMR_{O_2} to phenobarbital, specific differences are apparent. First, aged rats show more cerebral metabolic depression to large doses of phenobarbital than young rats. Second, young rats decrease CBF more than CMR_{O_2} during phenobarbital treatment, a relative vasoconstrictor effect which was not seen in senescent rats. These effects represent an age-induced change in responsiveness to the cerebral metabolic and cerebrovascular actions of phenobarbital that may be mediated by separate mechanisms.

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