

Circulating Catecholamines in Cats Before and After Lethal Head Injury (40419)

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With the recent development of an improved radio-enzymatic assay (1), it is possible to obtain reliable quantitative determinations of norepinephrine (NE), epinephrine (E), and dopamine (DA) using routine techniques. Normal circulating total catecholamine levels have been reported for man and for several common laboratory animals, and individual catecholamine levels have been reported for man (1) and the rat (2-4).

Lethal mechanical head injury by captive bolt results in a series of severe epileptiform convulsive seizures which are associated with increased discharge of sympathetic nervous system (5). Oscillations in systemic blood pressure following head injury are thought to be causally related to the gross lung injury which occurs in the rat (5) and could possibly play a role in the lung compliance and surfactant changes in the monkey (6) and the cat (7). Sarnoff and Sarnoff (8) showed that increased intracranial pressure in the dog resulted in increased systemic and pulmonary blood pressures with the consequent development of hemodynamic pulmonary edema (9). The theory of "neurogenic" pulmonary edema, referring to head injury-induced lung edema arising from neural influences within the lung itself has long been proclaimed (10) and disputed (8, 9). Whatever the mechanisms are which in fact do produce pulmonary injury following cerebral traumatization, it is likely that the sympathetic nervous system and probably the adrenal gland play an important role.

The purpose of the present study was to measure individual circulating catecholamine levels in the normal, conditioned, unanesthetized cat and to determine the effects of lethal mechanical head injury on these values. In order to avoid the possible influence of an anesthetic agent or problems attendant to an indwelling catheter in the cat, a technique was developed by which blood was withdrawn rapidly and painlessly from the hearts of calm, unstressed cats. Animals showing

any overt signs of stress, such as minor struggling or sudden movements, were placed in a separate category for analysis.

Methods. In eleven conditioned, unanesthetized, adult cats, a blood sample of 1-2 ml was very carefully and rapidly withdrawn by heart puncture. This procedure was perfected by handling the cats and avoiding the ribs, so as to minimize the stress, if any, to the cats so that, in most cases, the animals did not object, and in fact, gave no evidence of any awareness of the procedure. The cats which did respond by minimal struggling movements were placed in a separate "mildly stressed" group. Because evidence showed that a delay of 10 or more sec between the heart puncture and blood withdrawal occasionally did increase the catecholamine levels slightly, we withdrew the blood samples nearly instantaneously. Blood was collected in tubes containing 20 μ liters/ml of a solution of 90 mg/ml EGTA and 60 mg/ml reduced glutathione (catecholamines assay kit, Upjohn Diagnostics) and, after centrifugation, plasma was stored at below -25° to prevent breakdown of the catecholamines until assayed. After the control sample was withdrawn, the cat was exposed to sudden lethal mechanical head injury by means of a cartridge-fired bolt (Remington humane stunner, Model 412-A) designed for use as an approved and effective procedure in the abattoir industry. The round impact surface was 2 in. in diameter, had an effective mass of 3 lb. and traveled at an initial rate of 50 feet/sec. The instrument was hand-held against the occiput at an angle 45° from horizontal while the cat was in a normal, relaxed, standing position (6). Every blow was lethal and while none of the animals regained consciousness, we were prepared to administer lethal sodium pentobarbital at any initial signs of a pending return of normal reflexes. Immediately following the blow, while the animals were still convulsing, blood samples were taken from the heart at intervals of 5-15, 30-60, and 90-120 sec posttrauma-

tization.

Plasma levels of NE, E and DA were measured by a radioenzymatic assay (1) utilizing the catecholamines assay kit (Upjohn Diagnostics). All values for tests of statistical significance were compared to their corresponding normal controls using the Student's *t* test.

Results. The results from the present study show that lethal mechanical head injury increases circulating catecholamine levels in the cat by 10–100 times over control values. The mean circulating NE level in six unstressed control cats was 0.54 ± 0.07 (SE) ng/ml of plasma and 2.72 ± 0.35 in the three inadvertently mildly stressed controls ($P < 0.001$) (Fig. 1). Following lethal head injury during the 5–15 sec interval, the NE levels in three cats increased to 48.14 ± 23.56 ($P < 0.05$) followed by a decrease at the 30- to 60-sec interval to 25.22 ± 5.82 ($N = 6$) ($P < 0.001$). NE levels in seven cats then rose to 55.71 ± 23.58 at the 90- to 120-sec interval after this head injury ($P < 0.025$).

E and DA levels followed a somewhat similar trend. The mean unstressed control circulating E level was 0.26 ± 0.03 ($N = 6$) ng/ml compared to 1.23 ± 0.22 in the three slightly stressed controls ($P < 0.0025$) (Fig. 1). During the initial 5- to 15-sec interval following this head injury, the E level increased to 113.49 ± 41.26 ($N = 5$) ($P < 0.025$) followed by a decrease to 21.16 ± 6.10 ($N = 6$) ($P < 0.005$) at the 30- to 60-sec interval and a subsequent increase at 90–120 sec to 33.29 ± 10.30 ($N = 7$) ($P < 0.005$). The DA mean value for the 6 unstressed control cats was 0.22 ± 0.05 ng/ml and 0.87 ± 0.21 ($P < 0.01$) in the three slightly stressed cats, compared to 3.23 ± 0.44 ($N = 6$) at the 5- to 15-sec interval ($P < 0.001$), 1.96 ± 0.39 ($N = 7$) at the 30–60 sec interval ($P < 0.001$) and 2.80 ± 0.55 ($N = 8$) at the 90- to 120-sec interval ($P < 0.001$) (Fig. 1). All of the above mean values for NE, E and DA following this stress or head injury are significantly different by the Student's *t* test from their corresponding unstressed control values at least to the 0.05 level.

All variation in our sample size in the posthead injury groups was due to a breakdown in the assay as indicated by a smaller combined value for sample plus standard than for the sample alone.

Discussion. The results from the present

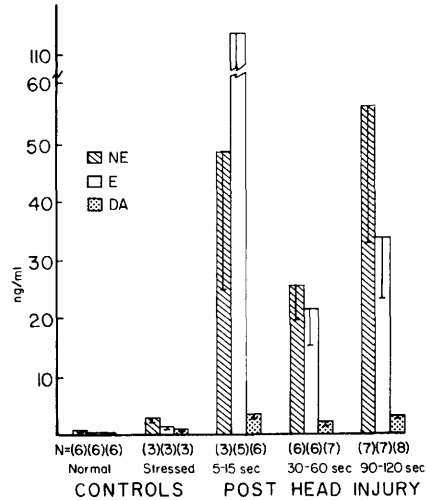


FIG. 1. Mean levels of catecholamines \pm SE in cats before and after lethal head injury. All values are significantly higher than their corresponding normal control values. Sample size is given in parentheses.

study give normal circulating catecholamine levels for the unanesthetized, unstressed cat. The results also show that lethal mechanical head injury results in a striking increase in the circulating levels of NE, E and DA within 15 sec following the blow. The normal circulating NE levels for the unstressed cat reported herein as 0.54 ng/ml are reasonably comparable to those reported for the rat to be 0.48 (4) and 0.15 ng/ml (2) as well as 0.63 ng/ml after manipulation and 0.23 ng/ml after halothan anesthesia (2). NE values following decapitation in the rat (2) were reportedly 13.20 ng/ml, which compares with our values of 48–56 ng/ml in the traumatized cat. In another recent study (3) NE levels in unstressed rats under halothane anesthesia were found to range from 0.82 to 1.00 ng/ml. E levels ranged from 0.37 (4) to 1.10 ng/ml (3) compared to our present value for E of 0.26 ng/ml. Circulating levels in human supine adults (1) were 0.28 ng/ml for NE, 0.023 ng/ml for E, and 0.034 ng/ml for DA. Using an indwelling catheter basal values for the cat for NE, E and DA were reported to be 0.61, 0.07 and 0.28 ng/ml (11) versus our values of 0.54, 0.26 and 0.22 ng/ml showing very close agreement for NE and DA and near basal levels for E.

The use of heart puncture technique for obtaining blood samples following head injury was very rapid and easy to perform in a

convulsing animal. We found that most of the conditioned cats permitted us to obtain an unstressed control sample provided that (a) the cats were handled gently before the heart puncture, (b) the needle did not touch a rib, (c) the blood sample was withdrawn very rapidly.

Mechanical head injury results in a massive sympathetic discharge as evidenced by epileptiform convulsive seizures, pupillary dilation, and a marked increase in blood pressure. The origin of the circulating catecholamines is attributed to discharge of sympathetic nerve endings and to release from the adrenal medulla. The biphasic response seen in Fig. 1 may be related to an ischemic response which peaks with the rise in blood pressure and is then followed by partial circulatory collapse with further catecholamine release. This increased sympathetic tone associated with a blow to the CNS has been described previously (9, 10) and the sympathetic causal relationship confirmed by the use of pharmacological blocking agents (5). Some recent evidence has suggested that alveolar surfactants may play a major role in the development of subsequent lung injury following CNS traumatization (6).

In conclusion the results from the present study show that near basal levels of NE, E and DA found in the cat in the present study were similar to those reported elsewhere for humans and rats and that a lethal blow to the head dramatically increased these values.

Summary. In 11 carefully conditioned, unanesthetized, relaxed, hand-held cats, blood samples were withdrawn instantaneously following heart puncture. Procedures were developed and practiced in order to avoid or greatly minimize any stress to the cats. Visible signs of very minor discomfort were present in only three cats while the others appeared to be unaware of the procedure. After these normal control samples were taken, the cats were exposed to sudden lethal mechanical head injury by means of a captive-bolt mech-

anism as is commonly used in the abattoir industry. Samples were taken rapidly from the heart at approximately 5- to 15-, 30- to 60-, 90- to 120-sec intervals following this head injury. Mean unstressed control values in six cats for norepinephrine, epinephrine and dopamine were 0.54, 0.26 and 0.22 ng/ml of plasma respectively. In the three cats which showed signs of mild discomfort, the values were 2.72, 1.23 and 0.87. However, following lethal head injury by 5-15 sec, these values for norepinephrine, epinephrine and dopamine rose to 48.14 ($N = 3$), 113.49 ($N = 5$) and 3.23 ($N = 6$) respectively. At 30-60 sec after this head injury, all of the catecholamine levels decreased but then subsequently rose again at the 90- to 120-sec interval. The results from the present study give normal feline circulating catecholamine values which show a striking increase following lethal mechanical head injury.

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