# The Avian Neurotransmitter[1] (37383)

PAUL D. STURKIE AND D. W. POORVIN
(Introduced by P. Griminger)
*Department of Environmental Physiology, Rutgers University, New Brunswick, New Jersey 08903*

Some investigators have reported higher levels of norepinephrine (N) than epinephrine (E) in avian blood and certain tissues (3, 5, 6) while others have not (2, 4, 7, 10). However, higher levels of E in the blood and heart do not necessarily indicate that E is the neurotransmitter, but may mean only that the latter is derived from the blood. It is well known that the myocardium can extract catecholamines from the blood. Sturkie *et al.* (10) have demonstrated that excitement, handling, anesthesia and method of collecting blood samples, factors which affect the release of catecholamines particularly from the adrenals, can influence blood and tissue levels significantly. These factors, therefore, may account in part for the discrepancies reported in the literature.

The ultimate test of a cardiac neurotransmitter is whether N or E is released after stimulation of the cardio-accelerator nerve (CA), and whether or not N or E is bound to the nerve terminals. Thus, one of the objectives of this experiment was to determine the nature of the neurotransmitter by stimulating the CA nerve in the isolated heart to determine whether N or E is released in amounts measurable by chemical means.

Another objective was to determine the effect of the released catecholamines on heart rate in isolated hearts uncomplicated by adrenal release of catecholamines, after CA nerve stimulation and before and after administration of adrenergic and cholinergic blocking agents.

*Materials and Methods. Isolated hearts.* Adult white Leghorn males were anesthetized with sodium pentobarbital (30 mg/kg) and heparinized with 400 units/kg. The thorax was opened and the heart removed quickly and the aorta attached to the nozzle of a Langendorff perfusion apparatus. The heart was perfused with Hank's solution [see Lockwood (9)] at a pressure sufficient to yield a flow rate of the effluent of about 30–40 ml/min. The temperature of perfusate was kept at 40° and a mixture of 95% $O_2$ and 5% $CO_2$ was continually bubbled through the solution. To record heart rates, a string from the apex of the heart was attached to a Grass force–displacement transducer.

In order to remove the cardioaccelerator nerves with the heart, they were first located in the intact heart and tied. Details on isolation and stimulation of these nerves and denervation have been described in detail by Tummons and Sturkie (11–13).

The stimulus parameters were 8 V and 20 Hz, with a pulse duration of 5 msec, provided by a Grass stimulator (54 GR).

Determination of N and E in the effluents of isolated hearts and heart tissue were based on the aluminum oxide–trihydroxyindole, fluorescent method of Anton and Sayre (1) as previously employed by Lin and Sturkie (7) and Sturkie *et al.* (10). In studying the release of N and E, the CA nerve was stimulated for 1–2 min and the effluent collected (30–60 ml) and analyzed for N and E. In some instances a larger volume of perfusate over a longer period of time was collected and N and E extracted.

The CA nerves of isolated hearts were stimulated under the following conditions. The right CA nerve was stimulated alone for periods of up to 1 min and heart rate recorded before and after stimulation. It was also stimulated after administration of propranolol at the previously established blocking dose of 200 ng/ml of perfusate (Hank's),

[1] Supported by GR 72-3 American Heart Association, New Jersey Affiliate.

TABLE I. Epinephrine (E) and Norepinephrine (N) Content ($\mu$g/g) of Chicken Hearts Perfused for 5 min (Means and Standard Errors).

| | Right atrium | | | Right venticle | |
|---|---|---|---|---|---|
| No. birds | N | E | No. birds | N | E |
| 7 | 0.52 ± 0.12 | 0.04 ± 0.09 | 5 | 0.36 | 0.06 |

and before and after administration of a blocking dose of atropine (50 ng/ml).

*Results. Release of N and E.* The concentration of N and E in the atria of the intact chicken heart averages about 0.96 and 1.5 $\mu$g/g of tissue, respectively, and the levels in the ventricles are 0.30 and 0.46, respectively (10). The effects of perfusion of isolated hearts for 5–10 min are shown in Table I where it is obvious that most of the epinephrine, but not norepinephrine, is washed out in the perfusate.

The effects of CA nerve stimulation on the release of N and E in isolated hearts are presented in Table II, where it is evident that appreciable amounts of N and practically no E are released on nerve stimulation, or the amounts released are beyond the limits of sensitivity of the method used. Actually, only 2 birds had even measurable amounts of epinephrine in the perfusate.

When stimulations of the CA nerve were employed intermittently over a period of 5–7 min and N and E extracted from about 200 ml of perfusate, greater amounts of N and only minute amounts of E were released.

The effects of CA nerve stimulation on heart rate are revealed in Fig. 1. The mean heart rate of group A before stimulation was 186 and after CA nerve stimulation 292 beats/min. This difference is highly significant. The prestimulation heart rate of the propranolol group, (B) was 176 and after CA stimulation 145. This represents a significant depression in rate and indicates a cholinergic component. When both propranolol and atropine are administered and both adrenergic and cholinergic effects blocked the heart rates before (186) and after stimulation (183) were not significantly different, which was not unexpected.

TABLE II. Levels of Norepinephrine (N) and Epinephrine (E) from the Effluents of Chicken Hearts at Different Times After Stimulation of Cardioaccelerator Nerve (Based on 9 Animals) (Mean and Standard Errors).[a]

| After | ng N/ml | ng E/ml |
|---|---|---|
| One minute* | 0.39 ± 0.09 | Trace |
| Two minutes** | 0.27 ± 0.08 | Trace |
| Five to seven minutes,[z] intermittently | 1.88 ± 0.46 | 0.08 ± 0.03 |

[a] N and E extracted from 30* and 60** ml of perfusate and from 200 ml perfusate.[z]

*Discussion.* The data demonstrate that when the isolated heart is perfused for 5–20 min, most of the epinephrine but not norepinephrine is washed out. This suggests that norepinephrine is tightly bound to the nerve terminals but that epinephrine is less tightly bound and probably localized in the chromaffin cells.

This assumption is born out by the fact that, following stimulation of the CA nerve, appreciable amounts of norepinephrine are released, but little epinephrine. Thus, the data prove that norepinephrine is the neurotransmitter in aves as it is in mammals. These and previous data from this laboratory (10) indicate that the high level of epinephrine reported in the blood and heart of aves is derived from non-neuronal sources, probably the adrenals.

The fact that heart rate is increased in the intact animal after stimulation of CA nerve even following depletion of neuronal catecholamines (14) indicates the release of an extra-neuronal catecholamine or another beta stimulator. Preliminary data on isolated avian hearts from this laboratory suggest that the extra-neuronal agent is epinephrine derived from the chromaffin cells of the heart, but this remains to be proven.

*Summary.* Stimulation of the cardioac-

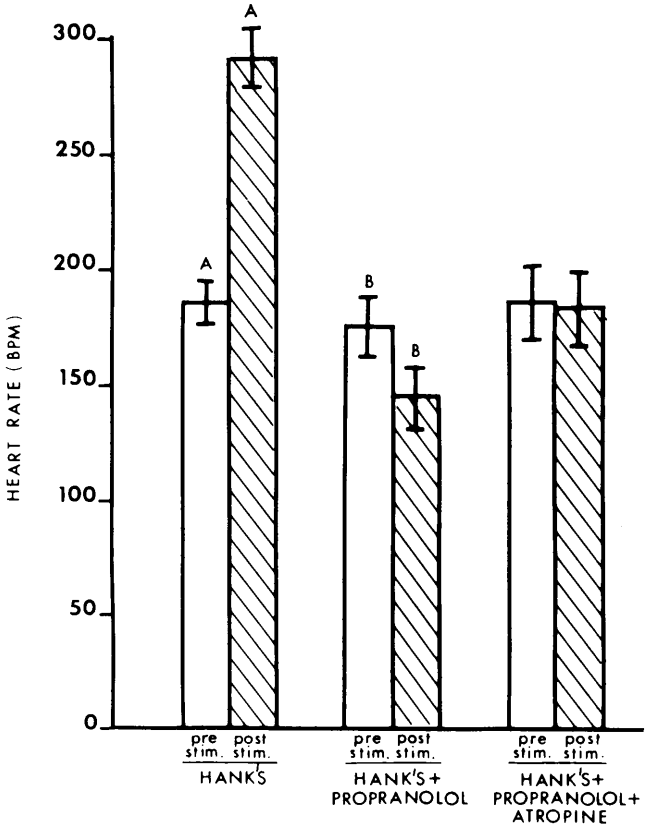


FIG. 1. Heart rate before (Pre Stim) and after (Post Stim) stimulation of cardioaccelerator nerve before and after propranolol (200 ng/ml of perfusate) and before and after atropine (50 ng/ml). Differences: A's $p < 0.01$, B's $p < 0.01$ each group based on 12 individuals.

celerator nerve (CA) in the isolated chicken heart causes the release of norepinephrine and little epinephrine and increases heart rate significantly, but after beta blockade heart rate was depressed indicating the release of cholinergic agent which could be blocked with atropine.

1. Anton, A. H., and Sayre, D. F., J. Pharmacol. Exp. Ther. **138**, 360 (1962).

2. Callingham, B. A., and Cass, R., *in* "Physiology of Domestic Fowl" (C. Horton-Smith and E. C. Amoroso, ed.). Oliver and Boyd, Edinburgh (1966).

3. Enemar, A., Falck, B., and Hakanson, R., Develop. Biol. **11**, 268 (1965).

4. Everett, S. P., and Mann, S. P., Eur. J. Pharmacol. **1**, 310 (1967).

5. Euler, von, U. S., *in* "Comparative Endocrinology" (U. S. von Euler and H. Heller, eds.), Vol. 2, p. 209. Academic Press, London (1963).

6. Ignarro, L. J., and Shideman, F. E., J. Pharmacol. Exp. Ther. **159**, 38 (1968).

7. Lin, Y. C., and Sturkie, P. D., Amer. J. Physiol. **214**, 237 (1968).

8. Lin, Y. C., Sturkie, P. D. and Tummons, J. L., Can. J. Pharmacol. **48**, 182 (1970).

9. Lockwood, A. P. M., Comp. Biochem. Physiol. **2**, 1241 (1961).

10. Sturkie, P. D., Poorvin, D. W., and Ossorio, N., Proc. Soc. Exp. Biol. Med. **135**, 267 (1970).

11. Tummons, J. L., and Sturkie, P. D., Life Sci. **7**, 377 (1968).

12. Tummons, J. L., and Sturkie, P. D., Amer. J. Physiol. **216**, 1437 (1969).

13. Tummons, J. L., and Sturkie, P. D., Comp. Gen. Pharmacol. **1**, 280 (1970).

14. Tummons, J. L., and Sturkie, P. D., Z. Vergl. Physiol. **68**, 268 (1970).

Received Nov. 21, 1972. P.S.E.B.M., 1973, Vol. 143.