Biochemical Pharmacology, 1967, Vol. 16, pp. 227–228. Pergamon Press Ltd., Printed in Great Britain.

# Brain levels of norepinephrine and serotonin in isolated rats receiving pargyline*

(*Received* 5 *April* 1966; *accepted* 1 *September* 1966)

RECENT studies[1, 2] indicate that alterations in brain levels of norepinephrine and serotonin occur in rats subjected to stress-inducing conditions such as electroshock and forced swimming. These studies suggest that changes in the levels of brain monoamines might be characteristic of stress and might therefore be expected to occur in other stress-inducing conditions such as chronic isolation. However, no measurements of brain monoamine levels have been reported for isolated rats. In the experiments to be described it was our object to determine whether changes in brain levels of norepinephrine and serotonin in isolated rats might be detectable.

When monoamine oxidase (MAO) is inhibited, there accumulate in the brain monoamines that might otherwise be metabolized.[3, 4] The present work shows that, when MAO is inhibited by pargyline, brain serotonin levels in rats living in isolation are higher than those in rats living in pairs.

## MATERIALS AND METHODS

Male albino rats of the Sprague–Dawley strain (Charles River), 6 weeks old and initially weighing about 150 g, were divided into four groups, two of which were housed in pairs in metabolism cages (18 × 18 × 24 cm). The remaining two groups were housed in partitioned cages, one rat on either side of the partition. All rats were injected daily from 9.00 to 10.00 a.m. and each was offered 18 g Purina laboratory chow per day at 5.00 p.m., with water *ad lib.* All animals were sacrificed by decapitation between 4.00 and 6.00 p.m. on the Day 32 of injection. The brains were removed and frozen at −10° for subsequent determination of serotonin and norepinephrine. Samples of blood were collected, and the serum was preserved at −10° for corticosterone assay. Serum corticosterone was analyzed by the fluorescence method of Guillemin *et al.*[5] Norepinephrine and serotonin were assayed in the same brain by the method of Mead and Finger.[6]

Pargyline HCl† was dissolved in a vehicle consisting of 5% (v/v) Carbowax 400‡ and 0·5% (v/v) benzyl alcohol in distilled water. This vehicle was used to solubilize other drugs in related experiments and was used in the present work in order to obtain comparable results. Pargyline HCl was administered in doses of 25 mg/kg/day, and the vehicle dose was 1 ml/kg/day. All injections were made intraperitoneally.

## RESULTS AND DISCUSSION

Related studies[7] carried out in our laboratory indicated that in rats receiving doses of 25 mg of pargyline HCl/kg daily, brain MAO activity was completely inhibited. Therefore, the observed increases in the levels of brain norepinephrine and serotonin (Table 1) were not unexpected. It was very interesting, however, to find that isolated animals receiving pargyline had 28·2 per cent more brain serotonin than paired animals receiving the same drug. In contrast, levels of brain norepinephrine in these two groups were not significantly different.

Increased production of corticosterone is a component of the isolation syndrome in rats[8] and may be considered a measure of stress in these animals. In our work, the efficacy of the 32-day isolation period as a stressor seems to be confirmed by the presence of twice as much serum corticosterone in isolated rats as in paired rats (Table 1). Furthermore, it is important to note that these corticosterone levels were increased by approximately the same amount in all isolated rats, whether they received pargyline or not. The suggestion that pargyline in itself had no effect on serum corticosterone levels is supported both by these data and by the failure of the drug to alter corticosterone levels in paired rats.

* A preliminary report of this work was presented at the meetings of the American Association for the Advancement of Science in Montreal, December 1964.

† Kindly supplied by Abbott Laboratories, Chicago, Ill.

‡ Polyethylene glycol 400, Union Carbide Chemicals Co., New York, N.Y.

It is also important to note that, after 2 weeks, the isolated, pargyline-treated rats exhibited behavioral peculiarities not unlike those observed by Hatch *et al.*[8, 9] in rats isolated for 13 weeks; e.g. a marked tendency to squeal and to bite while being handled. Such behavior was not observed to a significant degree in the other treatment groups. It is possible, then, to postulate that pargyline served to accelerate the onset of symptoms which usually appear only after longer periods of isolation.

TABLE 1. THE EFFECTS OF ISOLATION AND PARGYLINE TREATMENT ON THE LEVELS OF BRAIN NOREPINEPHRINE, BRAIN SEROTONIN, AND SERUM CORTICOSTERONE*

| Treatment | N | Norepinephrine (μg/g tissue) | Serotonin (μg/g tissue) | Corticosterone (μg/ml) |
|---|---|---|---|---|
| Vehicle-paired | 8 | 0·36 ± 0·04† | 0·47 ± 0·05 | 0·16 ± 0·03 |
| Vehicle-isolated | 12 | 0·38 ± 0·26 | 0·52 ± 0·08 | 0·35 ± 0·02 |
| Pargyline-paired | 8 | 0·84 ± 0·30 | 0·92 ± 0·24 | 0·15 ± 0·07 |
| Pargyline-isolated | 12 | 0·69 ± 0·25 | 1·18 ± 0·14 | 0·31 ± 0·03 |

| Statistical comparisons | *t*-Test: significance at indicated *P* values | | |
|---|---|---|---|
| | Norepinephrine | Serotonin | Corticosterone |
| Vehicle-paired vs. vehicle-isolated | N.S.‡ | N.S. | 0·01 |
| Pargyline-paired vs. pargyline-isolated | N.S. | 0·05 | 0·01 |
| Vehicle-paired vs. pargyline-paired | 0·01 | 0·01 | N.S. |
| Vehicle-isolated vs. pargyline-isolated | 0·005 | 0·01 | N.S. |

* Determinations were made after 32 days of treatment.
† Values are expressed as the mean ± S.D.
‡ No significant difference between groups ($P \leqslant 0{\cdot}05$).

In summary, the data in this report suggest that elevated levels of brain serotonin may be a component of the isolation syndrome in rats.

*Acknowledgements*—This work was supported by Research Grant MY-4435 and by predoctoral fellowships GM-21,141 (AMG), and 5 F1-MH 21,095 (ABM) from the National Institutes of Health.

*Department of Pharmacology,*
*College of Pharmacy,*
*University of Rhode Island,*
*Kingston, R.I., U.S.A.*

A. M. GUARINO*
J. A. ROSECRANS†
A. B. MENDILLO
J. J. DEFEO

* Present address—Section on Cellular Pharmacology, Laboratory of Chemical Pharmacology, National Heart Institute, Bethesda, Md.

† Present address—Department of Psychiatry, Yale University School of Medicine, New Haven, Conn.

## REFERENCES

1. D. X. FREEDMAN, *Am. J. Psychiat.* **119**, 843 (1963).
2. D. X. FREEDMAN and N. J. GIARMAN, in *EEG and Behavior* (Ed. G. H. GLASER), p. 198. Basic Books, New York (1963).
3. W. A. HIMWICH and E. COSTA, *Fedn. Proc.* **19**, 838 (1960).
4. A. PLETSCHER, *Pharmac. Rev.* **18**, 121 (1966).
5. R. GUILLEMIN, G. W. CLAYTON, H. S. LIBSCOMB and J. D. SMITH, *J. Lab. clin. Med.* **53**, 830 (1959).
6. J. A. R. MEAD and K. F. FINGER, *Biochem. Pharmac.* **6**, 52 (1961).
7. A. B. MENDILLO, A. M. GUARINO and J. J. DEFEO. Presented at A.Ph.A. Meetings, Dallas, April (1966).
8. A. M. HATCH, G. S. WIBERG, Z. ZAWIDZKA, M. CANN, J. M. AIRTH and H. C. GRICE, *Toxicol. appl. Pharmac.* **7**, 737 (1965).
9. A. HATCH, G. S. WIBERG, T. BALAZS and H. C. GRICE, *Science, N.Y.* **142**, 507 (1963).