J. Pharm. Pharmac. 1973, **25,** 164

LETTERS TO THE EDITOR

Catecholamine concentrations in discrete areas of the rat brain after the pre- and neonatal administration of phencyclidine and imipramine

The administration of centrally-acting drugs to pregnant rats has been shown to affect some aspects of the behaviour of the offspring (Werboff & Gottlieb, 1963). The primary effects are on activity and emotionality, though sodium bromide (Harned, Hamilton & Borrus, 1940), alcohol (Vincent, 1958) and barbiturates (Armitage, 1952) also produce learning deficits. In view of the probable relation between brain monoamines and behaviour, I have examined whether the pre- and neonatal administration of the psychotropic drugs phencyclidine and imipramine permanently affects catecholamine metabolism in the rat central nervous system.

Phencyclidine or imipramine was administered in the drinking water of mature Wistar rats (Tuck) of either sex. Ascorbic acid was included in the solutions and some rats received ascorbic acid only in solution. Solutions were freshly prepared every two days and were stored in air-tight amber glass bottles. Drug concentrations were increased over a period of weeks until behavioural disturbances became obvious, they were then reduced to and maintained at 200 mg litre^{-1}. After four weeks, during which food, fluid intake and body weight were monitored, animals were paired in individual cages (15 pairs per treatment). Two weeks later males were removed and the females continued to receive drug solution throughout pregnancy and the suckling period. Offspring were weaned at 21 days and thereafter received no drugs.

Male offspring, 4–8 a litter caged in randomly determined groups of 5, were killed by stunning and decapitation at 3, 6, or 9 months after weaning. Brains were rapidly removed and maintained at −20° until dissection which was on aluminium foil over an ice-salt mixture. To avoid contamination of regions of low catecholamine content by regions of high content, wherever possible, 100 mg samples were taken. In areas with total weight less than 100 mg, and for the "amygdaloid" region where no attempt was made to separate the nucleus from the surrounding tissue, the same weight (±5%) of tissue was taken from each brain. The brains were kept in a semi-frozen condition throughout. Separated areas were wrapped individually in aluminium foil and stored at −70° (for not more than 7 days) for catecholamine determination on two regions at a time, immediately before which they were weighed rapidly and homogenized in 4·0 ml of 0·01N hydrochloric acid with 0·4 ml of 10% ethylenediaminetetracetic acid (disodium, dihydrate) solution. The homogenates were centrifuged at 6000 rev min^{-1} for 20 min and a 2·0 ml aliquot of the clear supernatant taken for the determination of noradrenaline and dopamine by the method of Welch & Welch (1969) except that the volume of n-butanol used for the extraction was 8·0 ml which after mechanical shaking and centrifugation, was removed as completely as possible and shaken with 12·0 ml of n-heptane and 1·5 ml of 0·5M phosphate buffer (pH 7·3). Two 0·5 ml aliquots of the buffer were taken for the determination; tissue blank estimations were made on aliquots from a pool of the remaining buffer for each group. Normetanephrine concentrations were determined in another of the original acid aliquots exactly as described by Leonard & Tonge (1969). Fluorescence was read with a Perkin-Elmer MPF 3 spectrophotofluorimeter which gave readings in the middle range of the sensitivity scale with areas of low catecholamine content.

Table 1. *Noradrenaline, dopamine and normetanephrine concentrations in discrete areas from the brains of rats exposed to phencyclidine and imipramine during the pre- and neonatal periods.*

	None	Phencyclidine	Imipramine
Noradrenaline			
Cortex	1·56 ± 0·09	2·27 ± 0·08***	1·46 ± 0·12
Hippocampus	1·54 ± 0·06	1·48 ± 0·08	1·77 ± 0·12
Striatum	1·30 ± 0·07	1·03 ± 0·08*	1·25 ± 0·09
Thalamus	1·72 ± 0·09	1·83 ± 0·10	1·72 ± 0·12
Hypothalamus	10·6 ± 0·30	9·41 ± 0·25*	10·1 ± 0·32
Corpora quadrigemina	2·43 ± 0·13	2·43 ± 0·12	2·43 ± 0·12
Pons and medulla	4·14 ± 0·12	5·21 ± 0·13***	3·98 ± 0·14
"Amygdaloid"	1·72 ± 0·15	1·66 ± 0·12	1·72 ± 0·17
Dopamine			
Cortex	0·89 ± 0·04	1·18 ± 0·04***	0·79 ± 0·06
Hippocampus	1·14 ± 0·05	1·05 ± 0·04	1·18 ± 0·07
Striatum	37·9 ± 4·0	41·2 ± 5·2	29·6 ± 3·5
Thalamus	0·85 ± 0·07	0·88 ± 0·08	0·85 ± 0·06
Hypothalamus	0·90 ± 0·06	0·88 ± 0·07	0·82 ± 0·05
Corpora quadrigemina	1·83 ± 0·11	1·70 ± 0·10	1·76 ± 0·09
Pons and medulla	3·76 ± 0·20	5·10 ± 0·18***	4·12 ± 0·35
"Amygdaloid"	1·05 ± 0·08	1·01 ± 0·04	1·06 ± 0·06

Values for normetanephrine did not differ significantly from the control values of: Cortex, 0·71 ± 0·04; hippocampus, 0·97 ± 0·07; striatum, 1·99 ± 0·15; thalamus, 1·13 ± 0·12; hypothalamus, 9·73 ± 0·96; corpora quadrigemina, 1·69 ± 0·12; pons and medulla, 3·55 ± 0·20; "amygdaloid," 0·91 ± 0·10.

All values are the means of 5 determinations (made on discrete regions from 5 single brains), expressed as nmol g^{-1} ± s.e. Statistical significance (Student's t) is shown as *$2P = 0.05$, **$2P < 0.01$, ***$2P < 0.001$.

There were no statistically significant differences between catecholamine concentrations in brain regions from rats of different ages. The results presented in Table 1 are those from rats killed nine months after weaning.

Phencyclidine produced permanent effects on the concentrations of both noradrenaline and dopamine in discrete regions of the central nervous system while imipramine did not. Phencyclidine is a psychotomimetic drug which affects catecholamine metabolism in the brains of mature rats (Leonard & Tonge, 1969; Tonge & Leonard, 1972). That exposure of the pre- and neonatal rat to the drug results in apparently permanent changes in brain catecholamine concentrations, suggests that the developing brain may be particularly susceptible to phencyclidine. If this is so, and particularly if the same techniques can be used with other drugs, the developing brain may provide a useful tool for the detection of the neurochemical sites of action of psychotropic drugs.

The rats did not show any signs of disturbed behaviour which is perhaps surprising in view of the altered catecholamine concentrations observed, however, Werboff (1970) suggests that the behavioural effects of pre-natal drug exposure are often subtle.

School of Pharmacy,
Liverpool Polytechnic,
Byrom Street,
Liverpool, U.K.

SALLY R. TONGE

August 29, 1972

REFERENCES

ARMITAGE, S. G. (1952). *J. comp. physiol. Psychol.*, **45**, 146–52.

HARNED, B. K., HAMILTON, H. C. & BORRUS, J. C. (1940). *Am. J. med. Sci.*, **200**, 846–50.

LEONARD, B. E. & TONGE, S. R. (1969). *Life Sci.*, **8**, 815–25.

TONGE, S. R. & LEONARD, B. E. (1972). *Psychopharmacologia*, **23**, 86–90.

VINCENT, N. M. (1958). *Am. Psychologist*, **13**, 401–7.

WELCH, A. M. & WELCH, B. (1969). *Analyt. Biochem.*, **30**, 161–79.

WERBOFF, J. (1970). In: *Principles of Psychopharmacology*. pp. 343–53. London and New York: Academic Press.

WERBOFF, J. & GOTTLIEB, J. S. (1963). *Obst. Gynecol. Survey*, **18**, 420–3.

Some observations on the pulmonary artery of the guinea-pig

Qualitative as well as quantitative variations of drug actions on the pulmonary artery of several species have been previously reported with special interest in the biphasic response to acetylcholine (Gaddum & Holtz, 1933; Smith & Coxe, 1951). However, drug effects on the pulmonary artery of the guinea-pig have not been thoroughly documented and therefore the responses to several pharmacological agents on this blood vessel have been examined.

The blood vessel was removed from guinea-pigs (Hartley strain, 200–250 g) into Tyrode solution and cut spirally to produce a strip of 1·5–2 cm in length. The tissue was suspended in Tyrode solution (containing 17 mg^{-1} litre ascorbic acid), at 37°, aerated with 5% carbon dioxide in oxygen and allowed to equilibrate for 60 min. The initial tissue tension was 0·5 g and recordings of contractions and relaxations were measured isometrically using a pen recorder.

The blood vessel contracted to the following drugs (threshold ranges expressed as $\mu g\ ml^{-1}$ base for 8–10 experiments): histamine, 0·075–0·1; 5-HT, 5·0–6·0; acetylcholine, 4·5–5·5; carbachol, 30–35 and noradrenaline, 0·02–0·05. Contractions to acetylcholine produced a transient biphasic response (Fig. 1) unlike the other spasmogenic agents. This was increased in height, but not duration of contraction, by eserine salicylate ($10\ \mu g\ ml^{-1}$). Relaxation to lower doses of acetylcholine ($0{\cdot}2–1{\cdot}0\ \mu g\ ml^{-1}$) were produced on the artery previously contracted with noradrenaline in 4 of the 8 preparations examined. Acetylcholine-mediated relaxation was not affected by eserine salicylate ($10\ \mu g\ ml^{-1}$) in these experiments. Both contraction and relaxation produced by acetylcholine were completely abolished by atropine sulphate ($2\ \mu g\ ml^{-1}$).

The vasodilator response with low doses of acetylcholine and the vasoconstrictor response at higher concentrations confirms the findings on dog and cat pulmonary artery (Gaddum & Holtz, 1933) but is in contrast to the pure vasoconstrictor response to this drug in the same vessel of the calf (Eyre, 1971) and rabbit (Su & Beavan, 1965).

Isoprenaline (50 ng-$2{\cdot}5\ \mu g\ ml^{-1}$) had neither an effect on the uncontracted nor contracted (produced by carbachol) artery strip (10 experiments) but at doses $> 5\ \mu g\ ml^{-1}$ slow contractions were produced. This contractile response was abolished by pretreatment with doses of phentolamine mesylate ($0{\cdot}5\ \mu g\ ml^{-1}$ for 5 min; Fig. 1) and phenoxybenzamine hydrochloride ($0{\cdot}5\ \mu g\ ml^{-1}$ for 5 min) that abolished the response to submaximal concentrations of noradrenaline ($0{\cdot}1–0{\cdot}2\mu g\ ml^{-1}$) but not affected by propranolol hydrochloride ($1\ \mu g\ ml^{-1}$) or atropine sulphate ($1\ \mu g\ ml^{-1}$). None of these agents affected base-line tension nor responses to acetylcholine, histamine and 5-HT at these concentrations. Salbutamol ($5–150\ \mu g\ ml^{-1}$) had no effect on the uncontracted or contracted preparation (4 experiments). Theophylline ($> 200\ \mu g\ ml^{-1}$), dibutyryl adenosine 3′,5′-monophosphate ($> 250\ \mu g\ ml^{-1}$) and sodium nitrite ($> 15\ \mu g\ ml^{-1}$) relaxed the uncontracted artery and also relaxed