Short communications

Permanent alterations in catecholamine concentrations in discrete areas of brain in the offspring of rats treated with methylamphetamine and chlorpromazine

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Methylamphetamine hydrochloride (80 mg/l.) and/or chlorpromazine hydrochloride (200 mg/l.) have been administered in the drinking water of female Wistar rats during pregnancy and suckling. The offspring were weaned at 21 days and thereafter received no drugs. Nine months later, male offspring were killed and noradrenaline and normetanephrine concentrations were determined in eight discrete areas of the brains: neocortex, hippocampus, striatum, thalamus, hypothalamus, corpora quadrigemina, pons/medulla, and amygdala region. Both drugs appeared to have permanently altered catecholamine concentrations in several areas of the brain. There was evidence of antagonism between the effects of the two drugs in the hippocampus, striatum, thalamus, and corpora quadrigemina, where the individual drugs produced altered noradrenaline concentrations but a combination of the two had no effect.

Werboff & Gottlieb (1963) have described behavioural abnormalities in the offspring of rats treated with psychotropic drugs during pregnancy. Experiments have now been carried out to see if the administration of two psychotropic drugs during the pre- and neonatal periods might be related to permanent alterations in catecholamine metabolism in the central nervous system. The purpose of the experiments was not to investigate the way in which such effects might be produced, but rather to produce alterations in monoamine metabolism without the acute use of drugs.

Methods. — Chlorpromazine, methylamphetamine, or a mixture of the two drugs was administered, in the drinking water, to mature Wistar rats (Tuck) of either sex. Ascorbic acid was included in each solution and some rats received ascorbic acid only in solution. Solutions were changed every two days and were stored in air-tight amber glass bottles: no solution was kept for longer than two days. Drug concentrations were gradually increased over a period of weeks until behavioural disturbances became obvious, and were then reduced to restore normal behaviour. The final concentrations chosen were: methylamphetamine 80 mg/l., chlorpromazine 200 mg/l., and methylamphetamine 80 mg/l. + chloropromazine 200 mg/l. After a period of four weeks, during which food and fluid intake and weight gain were measured, animals were paired in individual cages. Two weeks after pairing, male animals were removed. Female rats continued to receive drug solution throughout pregnancy and the suckling period. The offspring were weaned at 21 days and thereafter received no drugs.

Male offspring were killed by stunning and decapitation 3, 6 and 9 months after weaning. The brains were removed as rapidly as possible and maintained at −20° C until dissection. The dissection procedure used was as follows: with the ventral surface uppermost, a cut was made through the brain just anterior to the point of emergence of the optic nerves. The separated portion of the brain contained the major part of the striatum, which was dissected free of other tissue. Still with the ventral surface of the brain uppermost, the hypothalamus was dissected out by making incisions to the level of the third ventricle. The brain was then turned over so that its dorsal surface was uppermost and the lateral areas containing the amygdala regions were removed. The cortex was then pushed forward and over the brain stem to expose the hippocampus on the inner surface. After the removal of the hippocampus, portions of each cerebral hemisphere were removed, care being taken not to include those parts of the basal ganglia surrounding the thalamus and enclosed by the cerebral cortex. The cerebellum was removed from the brain stem, and the latter was separated into the thalami, the corpora quadrigemina and the pons and medulla. Preliminary experiments showed that attempts to use complete anatomical regions often resulted

in the contamination of areas of low catecholamine content by areas of high content. To avoid this, only portions of each area were used: all the surrounding tissue, including the border areas between regions, was dissected away (except in the case of the 'amygdala region') so that a relatively pure sample of each area remained. Wherever possible, 100 mg samples were taken; in areas weighing less than 100 mg, and in the case of the 'amygdala region' when no attempt was made to isolate the amygdaloid nucleus from the surrounding tissue, the same weight of tissue was taken from each brain. The brains were kept in a semi-frozen condition throughout by performing the dissection on aluminium foil over an ice/salt mixture. Separated areas were wrapped individually in aluminium foil and stored at −70° C until catecholamine determinations could be carried out, then weighed rapidly and immediately homogenized in 4·0 ml of 0·01 N hydrochloric acid with the addition of 0·4 ml of 10% w/v ethylenediaminetetracetic acid (disodium, dihydrate) solution. The homogenates were centrifuged at $800 \times g$ for 20 min and two 2·0 ml portions of the clear supernatant were taken for the determination of noradrenaline and dopamine by the method of Welch & Welch (1969), and normetanephrine by the method of Anton & Sayre (1966) as modified by Leonard & Tonge (1969). Determinations were carried out on two areas at a time; remaining areas were stored at −70° C until immediately before

TABLE 1. *Noradrenaline, dopamine and normetanephrine concentrations in discrete areas from the brains of rats exposed to methylamphetamine (MA) and chlorpromazine (CPZ) during the pre- and neo-natal periods*

Drug	None	MA	MA+CPZ	CPZ
Cortex				
Noradrenaline	1·56±0·09	1·70±0·06	1·42±0·12	1·36±0·10
Dopamine	0·89±0·04	1·22±0·04†	0·93±0·05	0·74±0·06
Normetanephrine	0·71±0·04	0·98±0·04†	0·71±0·05	0·87±0·03*
Hippocampus				
Noradrenaline	1·54±0·06	1·89±0·06†	1·36±0·10	1·21±0·12*
Dopamine	1·14±0·05	1·03±0·04	1·11±0·07	1·01±0·06
Normetanephrine	0·97±0·07	1·29±0·06†	0·81±0·08	1·34±0·05†
Striatum				
Noradrenaline	1·30±0·07	1·11±0·08	1·26±0·09	0·89±0·07†
Dopamine	37·90±4·00	39·20±5·10	44·40±3·80	34·00±2·10
Normetanephrine	1·99±0·15	2·13±0·21	1·86±0·16	2·02±0·18
Thalamus				
Noradrenaline	1·72±0·09	1·44±0·08*	1·87±0·07	1·89±0·05
Dopamine	0·85±0·07	0·75±0·06	0·91±0·05	0·75±0·04
Normetanephrine	1·13±0·13	0·91±0·09	1·27±0·06	0·92±0·07
Hypothalamus				
Noradrenaline	10·60±0·30	13·40±0·28‡	8·88±0·30†	6·98±0·28‡
Dopamine	0·90±0·06	0·95±0·08	0·85±0·06	0·73±0·04
Normetanephrine	9·73±0·96	7·76±1·20	6·67±1·50	13·20±1·00
Corp. quad.				
Noradrenaline	2·43±0·13	2·85±0·12*	2·13±0·12	1·72±0·16†
Dopamine	1·83±0·11	1·70±0·05	1·57±0·12	1·44±0·08
Normetanephrine	1·69±0·12	1·26±0·15	1·74±0·16	2·09±0·11†
Pons/medulla				
Noradrenaline	4·14±0·12	4·38±0·13	5·27±0·15‡	4·61±0·12*
Dopamine	3·76±0·20	4·05±0·16	4·05±0·21	3·99±0·22
Normetanephrine	3·55±0·20	2·73±0·21*	3·61±0·24	3·99±0·22
Amygdala region				
Noradrenaline	1·72±0·15	— 1·56±0·15	1·54±0·16	1·36±0·14
Dopamine	1·05±0·08	1·09±0·04	1·08±0·05	1·02±0·07
Normetanephrine	0·91±0·10	0·83±0·08	0·81±0·07	0·87±0·07

All values are the means of 5 determinations, expressed as nmol/g±S.E.M. Statistical significance (Student's *t* test) is shown as: * $P<0·001$; † $P<0·01$; ‡ $P<0·05$.

use ; no area was stored for longer than one week, and homogenates were always used immediately after preparation.

Because of the small size of the tissue samples, the volume of n-butanol used for the extraction of noradrenaline and dopamine was reduced to 8·0 ml. After mechanical shaking and centrifugation, the butanol phase was removed as completely as possible and shaken with 12·0 ml of n-heptane and 1·5 ml of 0·5 M phosphate buffer (pH 7·3). Two 0·5 ml portions of the buffer were taken for the determination of noradrenaline and dopamine as described by Welch & Welch (1969) ; tissue blank estimations were performed on portions of a pool of the remaining buffer for each group. Normetanephrine concentrations were determined 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.

Results.—There were no statistically significant differences between catecholamine concentrations in brain areas of the experimental rats at different ages. The results shown in the Table are those obtained from rats killed nine months after weaning.

There were no obvious disturbances in the gross behaviour of the rats, but six out of fifty-two of the mature offspring from the chlorpromazine group died from an unexplained convulsive disorder and the rats exposed to methylamphetamine during development were more difficult to handle than offspring from the other groups. No systematic study of more subtle behavioural parameters was attempted. The behavioural disturbances reported by Werboff & Gottlieb (1963) were largely confined to differences in activity and emotionality, which would not be apparent except in specific test situations.

Discussion. — The administration of methylamphetamine and/or chlorpromazine during pregnancy and suckling produces changes in catecholamine concentrations in the brains of the offspring. These changes seem to be principally in noradrenaline concentrations, and the fact that normetanephrine concentrations are also altered suggests that the whole metabolism of noradrenaline may be disturbed. In at least some of the areas examined, methylamphetamine and chlorpromazine seem to have antagonistic effects: methylamphetamine treatment results in increased concentrations of noradrenaline in the hippocampus, hypothalamus and corpora quadrigemina, whilst chlorpromazine treatment causes decreased concentrations in these same areas and in the striatum. Methylamphetamine treatment produces decreased noradrenaline concentrations in the pons and medulla, whilst chlorpromazine treatment produces increased concentrations. The antagonism between the effects of the two drugs is particularly apparent in the hippocampus, striatum, thalamus and corpora quadrigemina, where the individual drugs produce altered noradrenaline concentrations but a combination of the two has no effect.

In these preliminary experiments, the rats were exposed to methylamphetamine and/or chlorpromazine throughout the developmental period ; it seems possible that more selective exposure might result in less widespread effects. Animals treated in this way might be useful in the study of the effects of changes in monoamine metabolism on behaviour.

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