THE PERSISTENT ANTAGONISTIC ACTION OF *N*-ALLYL-1-(*P-CYCLO*HEXYLOXYPHENYL)ETHYLAMINE TO ANALGESIC AGENTS

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Received November 5, 1957

N-Allyl-l-(*p-cyclo*hexyloxyphenyl)ethylamine, given one to two days beforehand, reduced the amount of l-(*p-cyclo*hexyloxyphenyl)ethylamine entering the adrenal glands of rats after an intraperitoneal dose by about 5 μg./g. without affecting non-specific accumulation. The effect was not observed with a closely similar, but analgesically inactive amine. The cerebral cortex acquired increased ability to accumulate the amine but heart remained unaffected. The reduced ability of the adrenal to accumulate the amine was restored at the same time that the analgesic antagonistic activity of the allylamine had begun to wane.

Antagonism of morphine analgesia by nalorphine was mimicked in rats by *N*-allyl-l-(*p-cyclo*hexyloxyphenyl)ethylamine[1]. The effect was slow to develop, taking some twelve hours, and then persisted for several days. A formal chemical resemblance to dibenamine, whose persistent adrenergic blocking action has been attributed to absorption by, and slow release from, body fat[2], prompted an investigation of the distribution and release of the amine in rat tissues, and a search for any changes that could parallel its prolonged effect.

METHODS AND MATERIALS

(1-^{14}C)-*N*-Allyl-l-(*p-cyclo*hexyloxyphenyl)ethylamine hydrochloride, m.p. 162–164° with 26,200 counts/min./mg., and the corresponding primary amine, m.p. 176–178°, with 30,200 counts/min./mg., both at infinite thickness using a G.E.C. EHM2 mica end window counter, were prepared by published methods[1,3]. Albino rat tissue samples were dried at 100° and powdered. They were counted at infinite thickness on polythene discs[4], recording 2,000–2,500 counts (approximate error 2 per cent) for any tissue showing marked activity. Where necessary kieselguhr was added to the powder, usually the adrenal, as an inert diluent to give sufficient mass for infinite thickness.

Table I shows that any deviation produced by this procedure was about the same as the counting error. Animals were killed and bled out by excision of the heart under ether anaesthesia.

RESULTS

Distribution of the labelled allylamine in male rat tissues at ten minutes after giving 20 mg./kg. intraperitoneally or 10 mg./kg. intravenously resembled that of labelled 1-(*p-cyclo*hexyloxyphenyl)-ethylamine found in previous experiments[3]. Perirenal fat gave low counting rates, approximately three times background and equivalent to 5–10 μg./g. tissue.

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There was little activity in gastrocnemius muscle, plasma or whole blood. One hour after the dose the tissue concentrations were little changed, but only traces of activity remained in the nineteen tissues examined at twenty-four hours after the dose. Rather more than half the injected counts appeared in urine within twenty-four hours but little further

TABLE I

THE EFFECT ON COUNTING RATE OF DILUTION OF TISSUE SAMPLES WITH KIESELGUHR

Tissue	Dilution per cent	Observed count/min. ± S.D.	Expected count/min.
Kidney	0	288 ± 6	—
	40	178 ± 4	169–176
	60	115 ± 5	112–118
Cerebral cortex	0	198 ± 5	—
	50	113 ± 5	96–102
Heart	0	218 ± 5	—
	50	120 ± 5	106–116

excretion occurred by this route. Faeces were not examined. The only tissues found to show evidence of residual activity were those of adrenal and kidney. After lapse of four days after the dose, these tissues, when dried, gave about two counts/min. above a background of 8–10 counts/min.

The allylamine (20 mg./kg. intraperitoneally) or dibenamine (20 mg./kg. intraperitoneally) given two days previously, or nalorphine (20 mg./kg. intraperitoneally) given fifteen minutes previously had no obvious influence on the amount of 1-(*p-cyclo*hexyloxyphenyl)ethylamine entering tissues,

TABLE II

THE INFLUENCE OF ANTAGONISTS ON THE DISTRIBUTION OF 1-[^{14}C]-1-(*p-cyclo*HEXYLOXYPHENYL)ETHYLAMINE IN RAT TISSUES EXPRESSED AS THE RATIO TO CEREBELLAR CORTICAL CONCENTRATION

Tissue	Control Ratios	Allylamine 1 day	Allylamine 2 days	Allylamine 3 doses at 6, 4 and 2 days	Dibenamine 2 days	Nalorphine 15 mins.
Cerebral cortex	1·60 ± 0·05 (9)	1·80*	1·65	1·82*	1·83*	1·50
Thalamus	0·99 ± 0·12 (5)	1·23	1·11	—	1·15	—
Hypothalamus	0·80 ± 0·11 (4)	0·94	0·84	—	1·04	0·81
Dorsal root ganglia	0·47 ± 0·07 (4)	0·45	—	—	—	0·33
Adrenal	3·58 ± 0·32 (6)	2·63*	2·78*	2·54*	2·82*	2·90*
Thyroid	1·90 ± 0·57 (6)	—	1·93	—	1·14	2·32
Heart	1·85 ± 0·26 (6)	—	1·87	1·42	1·42	—

Doses of all drugs were 20 mg./kg., i.p. Rats were killed ten minutes after the dose of amine. Antagonists were given at times stated before the amine. Number of results for estimation of control ratios in parentheses. Allylamine = *N*-Allyl-1-(*p-cyclo*hexyloxyphenyl)ethylamine.

* Indicates ratios falling outside two S.D. of controls.

as assessed at ten minutes after dosage, when compared with previous results in the same strain of rat[3]. When the tissue distributions were expressed relative to a reference tissue however, a limited disturbance in the adrenal and cerebral cortex became apparent. Since plasma activity was always too low to count accurately and gave widely divergent ratios,

cerebellar cortex was chosen as the reference tissue. This could be reasonably expected to have little bearing on development of analgesia. Table II shows that in spite of considerable variation in tissue activities between individual rats (cerebellar cortical concentrations varied from 20–45 μg./g. wet tissue: mean, 31·4 $\pm$ 9·1 S.D.), the ratios to cerebellar cortical concentrations were reasonably constant in animals without drug pretreatment, for the tissues studied. Cerebellar cortical concentrations in rats previously given allylamine were rather higher but not significantly so (range 17–56 μg./g. wet tissue: mean, 41·2 $\pm$ 10·0 S.D.). In three

TABLE III

WET: DRY RATIOS OF RAT TISSUES AFTER TREATMENT WITH PHENYLETHYLAMINES

Tissue	l-(*p-cyclo*Hexyloxyphenyl)-ethylamine		*N*-Allyl-l-(*p-cyclo*hexyloxy-phenyl)-ethylamine
	After saline (6)	24 hr. after *N*-allylamine (5)	(4)
Cerebral cortex	4·96 $\pm$ 0·12	4·90 $\pm$ 0·13	4·89 $\pm$ 0·07
Cerebellar cortex	4·53 $\pm$ 0·06	4·50 $\pm$ 0·09	4·49 $\pm$ 0·07
Heart	4·12 $\pm$ 0·11	4·18 $\pm$ 0·14	4·16 $\pm$ 0·09
Adrenal	3·36 $\pm$ 0·14	3·31 $\pm$ 0·23	3·29 $\pm$ 0·18

Number of results used for estimation of control ratios in parentheses.
Doses were 20 mg./kg., i.p.
Tissues were taken ten minutes after the last dose of amine.

experiments under slightly different conditions (see Table II) the adrenals gave a ratio between two and three standard deviations lower than the controls. Dibenamine or nalorphine pretreatment in single experiments had a similar effect. Conversely the ratio to cerebral cortex tended to increase. Heart, thyroid, and a few gross dissections of the central nervous system showed no comparable change. The effect was not traceable to a fluid shift in the tissues. In spite of a marked increase in haematocrit after the amine there was little change in this strain of rat in the wet: dry ratios between treated and control animals (Table III).

To confirm the result, sixteen female albino rats of a different strain, weighing 225 g., were divided into two equal groups. Females were chosen since their adrenals are larger than in males. One group received the allylamine, 20 mg./kg. intraperitoneally, on each of two successive days, while the other group received saline. One pair from each group received labelled 1-(*p-cyclo*hexyloxyphenyl)ethylamine, 20 mg./kg. intraperitoneally, at 1, 2, 7 and 14 days after the last dose of allylamine. They were killed ten minutes after the dose, and the adrenals, apex of the heart, cerebral cortex and cerebellar cortex dissected rapidly and prepared for counting. A scintillation assembly was used instead of the mica end window counter. There was rather greater variation in the wet: dry ratios of the tissues compared with the previous result and a clearer picture emerged by calculating ratios on a dry weight basis. Some justification for this procedure can be derived from the following points which indicate that the amine is mainly associated with formed elements of the tissues rather than dissolved in cellular fluids. It was not possible to derive a reasonably

constant ratio by comparison with plasma activity and the amine virtually disappeared from blood within 2–5 minutes after intravenous injection. It was not absorbed from a subcutaneous depot, the tissue blackening and eventually sloughing as a hard mass, an observation consistent with the failure to obtain a subcutaneous LD50 value[5]. This absorption by tissue to give a spurious concentration gradient was observed *in vitro* when slices

TABLE IV

RESTORATION OF CONCENTRATION RATIO BETWEEN TISSUE AND CEREBELLAR CORTEX FOR 1-(*p-cyclo*HEXYLOXYPHENYL)ETHYLAMINE AFTER PRETREATMENT WITH THE CORRESPONDING *N*-ALLYLAMINE

Tissue	1-(*p-cyclo*Hexyloxyphenyl)ethylamine					1-(*p-iso*Propyloxyphenyl) ethylamine	
	Control Ratios ± S.D.	1 day	2 days	7 days	14 days	Control Ratios ± S.D.	1 day
Cerebral cortex	1·69 ± 0·12 (4·88 ± 0·09)	1·88 (4·96)	1·65 (4·74)	1·78 (4·90)	1·65 (4·99)	1·61 ± 0·02	1·70 ± 0·27
Heart	1·62 ± 0·15 (3·99 ± 0·12)	1·62 (4·04)	1·62 (3·96)	1·54 (4·01)	1·72 (4·15)	0·83 ± 0·12	0·91 ± 0·51
Adrenal	2·80 ± 0·30 (3·22 ± 0·16)	1·94 (3·09)	2·52 (3·12)	2·73 (3·15)	2·66 (3·33)	1·36 ± 0·07	1·39 ± 0·54

Ratios are referred to dry weights of tissue. In the treated series they are the mean of two results. Control ratios were estimated in a group of eight rats. Treated rats received the allylamine on two successive days before receiving the primary amine. Times refer to lapse after the last dose of allylamine. All doses were 20 mg./kg., i.p. Tissue wet : dry ratios are in parentheses.

of cerebral cortex took up the amine from salines independent of interference with energy generating processes by addition of metabolic inhibitors[6]. Table IV shows that the ratios for adrenal and cerebral cortex had the expected disturbance at one day after completing the allylamine dosage, that the cerebral cortex had recovered within two days, and the adrenal within seven days. The chemically similar but analgesically inactive 1-(*p-iso*propyloxyphenyl)-ethylamine[5] revealed no similar disturbance in two groups of four animals tested at one day following treatment with the allylamine. The heart remained unaffected in each instance.

DISCUSSION

The prolonged effect of the allylamine could not be related to absorption and retention by any of nineteen representative rat tissues, nor would this hypothesis explain the relatively long lag in onset of analgesic antagonism in this species. Agarwal and Harvey[7] thought that the prolonged effect of dibenzyline, a dibenamine-like drug, was not explicable on this basis. Though there was no good evidence for retention of the allylamine in tissues for periods exceeding twenty four hours, with the doubtful exceptions of adrenal and kidney, the method of assay was not sufficiently sensitive to detect amounts that could well have physiological importance. The concentrations required to give a count of twice background exceeded 3 μg./g. wet tissue, equivalent to 0·01 μmole/g. Moreover activity could be mediated by breakdown products not incorporating the labelled atom. Nevertheless, ignoring the last possibility, if it be assumed that the fall in ratio for the adrenal arises by blockade of specific receptors, an effect

being obscured by non-specific accumulation, then the amount of allylamine so associated at twenty four hours after the dose, as calculated from the observed decrease in ratio, should be about 5 μg./g., corresponding to a count roughly three times background and therefore easily detectable. Present results seem more consistent with a hypothesis that the amine alters tissues in the adrenal and possibly the cerebral cortex without retention to produce a slowly reversible change. The fair parallel between the restoration of the normal concentration ratio in the adrenal and return of sensitivity to the analgesic activity of the primary amine[1] suggests that these changes are related in some obscure manner to generation of analgesic antagonism. Possible reasons for an increased ratio in the cerebral cortex cannot be profitably discussed but it is of interest that the allylamine in no way antagonised the depression of spontaneous activity induced in rats by the analgesic primary amine.

Acknowledgements. Defrayment of the major cost of this investigation by a grant from the Medical Research Council and the assistance of Miss R. E. Segull are gratefully acknowledged. The author is indebted to Dr. W. G. Duncombe for arranging for the counting of several tissues, and to Mr. R. A. Nightingale for their preparation.

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