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TISSUE DISTRIBUTION OF α-^{14}C-LABELLED ALKYLOXY α-PHENYLETHYLAMINES IN RELATION TO ANALGESIC ACTIVITY

BY

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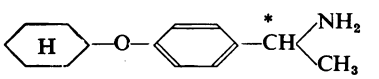
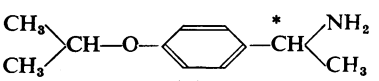
Minor changes in chemical structure could influence the physiological response to a drug by causing changes in those physical properties which determine access to susceptible tissues. The tissue distributions of ^{14}C-labelled *p-cyclo*hexyloxy-α-phenylethylamine (I) and *p-iso*propyloxy-α-phenylethylamine (II) have been studied with this concept in mind, since I has morphine-like properties not possessed by II (McCoubrey, 1953). The α-C-atoms (marked with an asterisk in formulae I and II) were the labelled atoms.



(I)



(II)

METHODS

The amines were given as the hydrochlorides in water. The doses (40 mg./kg. i.p. and 10 mg./kg. i.v.) were sufficient for I, but not for II, to induce well-marked analgesia (M.E.D. 10–15 mg./kg. i.p.). Animals were killed by excision of the heart under ether anaesthesia (3 minutes) unless stated otherwise. Tissues were always dissected in the same order. There was little change in tissue concentration during thirty minutes after exsanguination (Brierley, unpublished). Visceral tissues were removed rapidly after intraperitoneal injection to avoid an observed *post-mortem* absorption of drug from the peritoneal cavity. To obtain sufficient tissue, six male albino rats (300–350 g.) were used for each time interval. Approximately equal amounts of any one tissue were taken from each animal and pooled. Rabbits of one litter were used for each drug, two animals per time interval. The analgesia due to I was sufficient to allow killing, without anaesthesia, by medullary section through the atlanto-occipital membrane, sometimes after withdrawal of cerebrospinal fluid. Ether was required for II. The monkey (*Macaca mulatta*, ♂, 3.5 years) was lightly anaesthetized (ethyl chloride, then ether) before giving I into the saphenous vein.

Definition of Tissues.—Meninges and vessels were removed as completely as possible. *Cerebral cortex*—thin slices excluding visible white matter. *Subcortical white matter*—substantial contamination with grey matter is inevitable in small animals. Monkey corpus callosum is essentially pure white matter. *Thalamus*—isolated by coronal cuts at the interventricular foramen and habenula, laterally by an oblique cut at the junction with the internal capsule, inferiorly at the hypothalamic sulcus. *Hypothalamus*—from optic chiasma anteriorly to the mammillary bodies posteriorly and extending superiorly to the hypothalamic sulcus. *Cerebellum*—midline vermis with white core removed. *Medulla*—from auditory tubercle to obex with removal of area postrema. *Spinal cord*—cervical or lumbo-sacral enlargements. In certain cases cervical cord grey matter was carefully dissected from the white matter. *Dorsal root ganglia*—lumbo-sacral and cervical ganglia were pooled from rats, but were treated as separate groups in rabbit and monkey. The ventral roots were removed. Peripheral nerve and dorsal root were removed as close to the ganglion as possible. *Sympathetic ganglia*—superior cervical and stellate ganglia. *Adrenals*—rat adrenals were decapsulated. Rabbit adrenals were cut in three. The two outer thirds ("cortex") were assayed separately from the inner third.

Radioassay.—All tissues were dried at 60° C. 50 or 30 mg. of powdered tissue were assayed as "infinite thickness" samples (Popják, 1950) under a G.E.C. EHM2 mica window (1.9 mg./cm.^{2}) counter. The counting efficiency by comparison with a standard ^{14}C-polythene disc was 1.9%. Where insufficient dry tissue was available, kieselguhr was added and thoroughly mixed to make 30 mg. A linear relation between such dilution and activity was found in trial assays with various tissues. 2,000 counts, including background (10–13 counts per minute), were recorded for most tissues (error not greater than ±4.5%). 1,000–1,500 counts were recorded for tissues with low activity

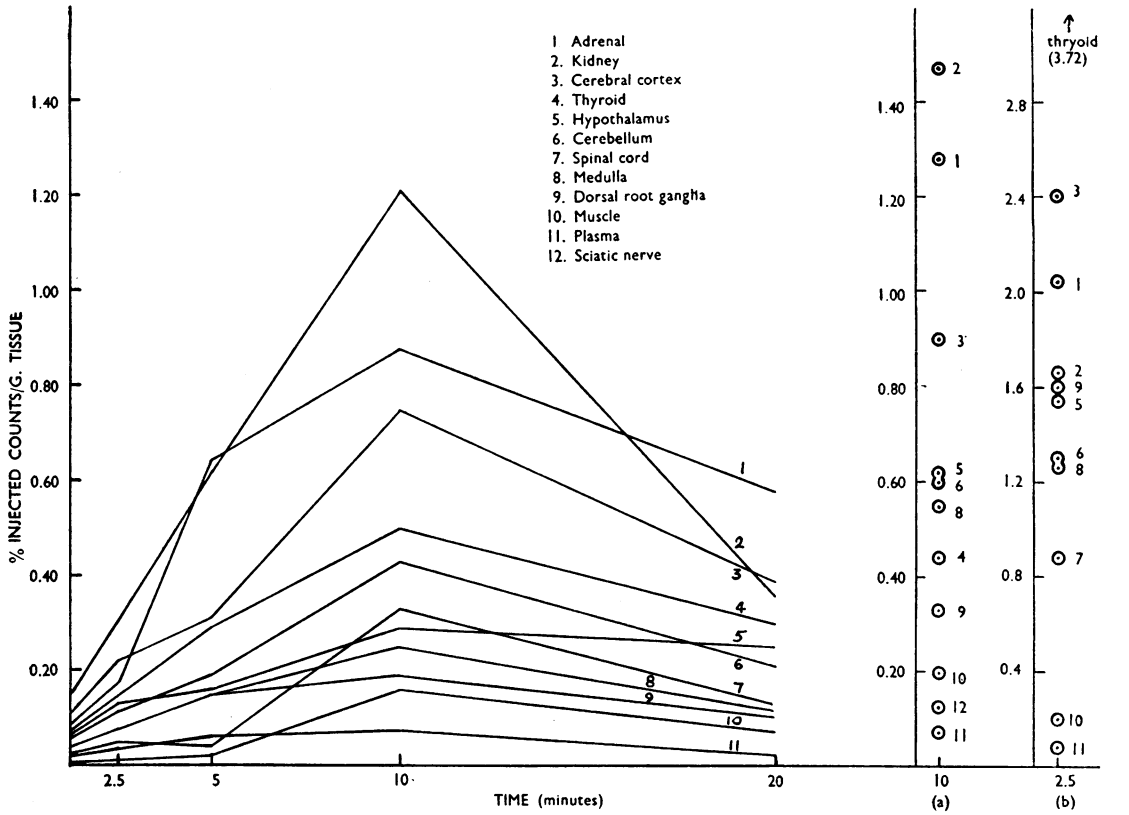


FIG. 1.—Rat tissue concentrations at various times after *p-cyclo*hexyloxy-α-phenylethylamine hydrochloride (40 mg./kg. i.p.). The ordinates represent percentage of injected counts per animal per gramme wet tissue. (*a*) Gives the tissue activities of *p-iso*-propyloxy-α-phenylethylamine under comparable conditions at ten minutes. (*b*) Gives the tissue activities at 2½ minutes after the *cyclo*hexyl ether (10 mg./kg. i.v.).

error not greater than ±13%). Tissue activities were calculated as percentage of injected counts per animal per g. wet tissue.

RESULTS

Tissue concentrations of I and II in the rat at various times after 40 mg./kg. intraperitoneally are given in Fig. 1, together with the concentrations of I at 2½ minutes after 10 mg./kg. intravenously. Results for the rabbits, which received 10 mg./kg. of I or II intravenously, are given in Fig. 2. These results are for one of two rabbits per time interval except where pooling of tissue was necessary. No significant difference in important tissues was observed in such pairs. Table I gives the concentration of I in monkey tissue five minutes after receiving 10 mg./kg. intravenously.

There was only low activity in muscle, liver, sciatic nerve, testis, trigeminal ganglion, and pituitary in both rat and rabbit. There was no activity in cerebrospinal fluid.

TABLE I

RADIOACTIVITY OF MONKEY TISSUES FIVE MINUTES AFTER *p-CYCLO*HEXYLOXY-α-PHENYLETHYLAMINE HYDROCHLORIDE (10 mg./kg.) INTRAVENOUSLY

Activity is expressed as the percentage of injected counts per gramme wet tissue.

Tissue	Activity	Tissue	Activity
Sensory cortex ..	0·109	Dorsal nerve root	0·016
Motor ,, ..	0·113	Dorsal root ganglion (cervical)	0·029
Visual ,, ..	0·110	Dorsal root ganglion (lumbar)	0·040
Frontal ,, ..	0·109	Dorsal root ganglion (sacral)	0·029
Cerebellar ,, ..	0·099	Sympathetic ganglion	0·075
Olfactory lobe ..	0·064	Sciatic nerve	0·006
Ventral thalamus ..	0·113	Saphenous nerve	0·013
Dorsal thalamus ..	0·095	Kidney cortex	0·338
Hypothalamus ..	0·062	Adrenal	0·333
Medulla	0·076	Pituitary	0·193
Corpus callosum ..	0·012	Thyroid	0·068
Spinal cord		Muscle	0·016
,, ,, (lumbar)	0·062	Liver	0·020
Spinal cord (cervical grey matter) ..	0·100	Whole blood	0·0047
Spinal cord (cervical white matter) ..	0·018	Plasma	0·0060

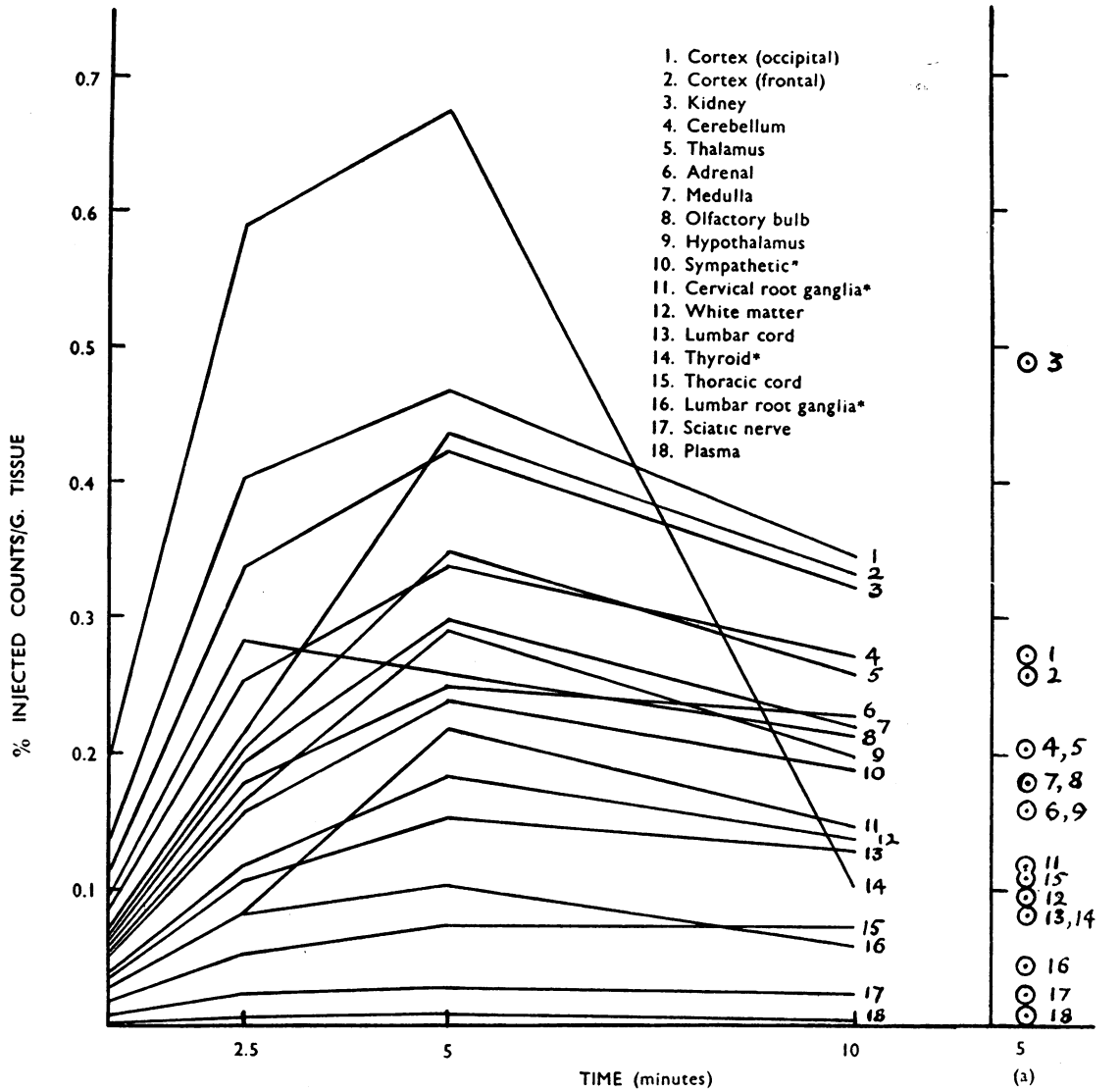


FIG. 2.—Rabbit tissue concentrations at various times after *p-cyclo*hexyloxy-α-phenylethylamine hydrochloride (10 mg./kg. i.v.). * Indicates where tissues were pooled from two animals. (*a*) Gives the tissue activities of *p-iso*propyloxy-α-phenylethylamine under comparable conditions at five minutes.

DISCUSSION

I and II, like most basic substances, readily penetrate nervous tissues (Friedemann, 1942). Table II clearly shows that pharmacological differences between these amines cannot be explained in terms of access to tissues. These amines have no markedly different preferences for nervous tissues, and II, which is not analgesic in the rat, penetrates all rat tissues examined more readily than I. The converse applies to the rabbit, but no pharmacological data with respect to these substances are available for this species. The tissue concentration-time curve (Fig. 1) is similar to the analgesic time-action curve for I in the rat and does not correspond to the time course of respiratory depression (McCoubrey, 1953). The ephemeral action of I is probably due to rapid elimination by the kidney. It was not possible to show any marked plasma activity, even after intravenous dosage, and the amines appear to be rapidly con-

TABLE II

COMPARATIVE TISSUE CONCENTRATIONS OF *p-CYCLO*-HEXYLOXY (I) AND *p-ISO*PROPYLOXY-α-PHENYLETHYL-AMINE (II) IN RAT AND RABBIT

Activities are calculated from $\frac{a \times n}{p \times N}$ where a = counts per gramme wet tissue, n = number of animals, p = counts per mil. in plasma. N = total injected counts for the group. The plasma activities (% injected counts per animal per gramme wet tissue) were respectively: rat: I, 0.071, II, 0.075; rabbit: I, 0.010, II, 0.013

Tissue	Rat			Rabbit		
	10 min. after 40 mg./kg. i.p.			5 min. after 10 mg./kg. i.v.		
	I	II	Ratio	I	II	Ratio
Cerebral cortex ..	10·6	13·5	1·27	—	—	—
,, occipital ..	—	—	—	47·0	20·6	0·45
,, frontal ..	—	—	—	43·4	20·4	0·47
Hypothalamus ..	4·2	8·2	1·95	29·8	12·4	0·42
Thalamus	—	—	—	34·6	15·6	0·45
Cerebellum	6·1	8·0	1·31	33·8	15·6	0·46
Medulla	3·5	7·3	2·10	30·0	13·8	0·46
Olfactory bulb ..	—	—	—	25·0	13·9	0·56
Dorsal root ganglia ..	2·8	4·4	1·58	—	—	—
,, lumbo-sacral ..	—	—	—	10·5	3·5	0·38
,, cervical ..	—	—	—	15·6	6·6	0·42
Sympathetic ganglia ..	—	—	—	24·2	6·6	0·27
Adrenal	12·4	17·0	1·37	23·2	12·4	0·54
Thyroid	7·0	5·9	0·84	67·5	8·8	0·13
Kidney	17·1	19·6	1·15	42·5	38·0	0·89
Muscle	2·2	2·6	1·18	6·8	4·4	0·65

centrated in nervous tissue. With the exception of rat and rabbit (but not monkey) cerebral cortex, there was no regional variation within grey matter when allowance was made for white matter contamination. The low activity in sympathetic and dorsal root ganglia was certainly due to low cell content, since in typical rat lumbo-sacral and cervical ganglia it was shown by projection of Nissl stained serial sections on to paper that the proportion of cells to fibres was 35 and 47% respectively (excluding ventral roots). In spite of the solubility of these amine hydrochlorides in non-polar solvents (McCoubrey and Iyengar, 1951) penetration into white matter was quite low.

It is not possible at present to relate the selective concentration in rat or rabbit cerebral cortex to analgesic activity. Similar selective localization does not occur in the monkey and in this animal there is no preference for either sensory or frontal cortex. There is little evidence that pain sensation is normally projected on to the cerebral cortex even in man. Penfield (1947) has shown that electrical stimulation of exposed human cerebral cortex in conscious subjects evokes no painful sensation, and, while section of frontal lobe tracts (lobotomy) relieves the anxiety associated with pain, it apparently does not reduce pain sensation, and indeed the threshold for experimental pain may be reduced (Chapman, Rose, and Solomon, 1948). In isolated cases excision of sensory cortex can relieve pain of long standing (Lewin and Phillips, 1952), but in cases of intractable pain the most effective procedure appears to be section of spinothalamic tracts (Falconer, 1953).

There are indications that some, but certainly not all, aspects of analgesic mechanism are mediated at a spinal level. Spinal structures were therefore examined in some detail, especially the dorsal root ganglia, since all afferent sensory impulses traverse these ganglia. Brierley (1951, 1952) has shown that they concentrate a number of ions, and, although there are no synapses here, a brief delay occurs in impulse transmission across the ganglia (Dun, 1951), which could indicate actual participation by the cells in transmission. In the dorsal horn grey matter, Pearson (1952) finds histologically that slow pain fibres make their synapse in the dense neuropil of the *substantia gelatinosa Rolandi*, whereas fast pain fibres by-pass this region to synapse in the *nucleus proprius*. This is of interest in view of Cushny's remark (1941) that "sudden shocks" (fast pain?) are distinguishable under morphine while pain (slow pain?) is under control. Further, spinal animals react equally well in analgesic assays which measure depression of avoidance reflexes (Bonnycastle and Leonard, 1950; Houde and Wikler, 1951). The appreciable concentration of drug, however (approx. 10^{-7} moles per g. tissue), which appeared at all cell stations on the pain pathway, viz., dorsal root ganglia, spinal cord grey matter, medulla and thalamus, with little in the interconnecting fibre tracts and peripheral nerve, suggests that the action of analgesics may be an attenuation of pain impulses at the successive cell stations.

The difference in pharmacological response between I and II had suggested that some differences in localization might occur in the nervous system. Marked divergence was only observed in the adrenal and thyroid glands. Mere concentration does not necessarily imply a role for these glands in analgesia; there is considerable evidence that their secretions markedly influence the action of analgesics, but discussion can only be speculative in the absence of experimental results.

CHEMICAL SECTION

The route previously used for synthesis of alkyloxy-α-phenylethylamines was not wholly suitable for ^{14}C-labelling. *p-Cyclo*hexyloxyphenyl magnesium bromide was carbonated to give the carboxylic acid, whence the lithium salt and lithium methyl (Gilman and Van Ess, 1933) gave *p-cyclo*hexyloxyacetophenone. The ketoxime was reduced to the amine as previously described (McCoubrey and Iyengar, 1951). The *iso*propyl ether was prepared similarly.

p-*Bromophenyl* Cyclo*hexyl Ether.*—*p*-Bromophenol (20 g.) and *cyclo*hexyl bromide (57 g.) by the method described (McCoubrey and Iyengar, 1951) gave the *ether* (10.9 g.), b.p. 115–117°/0.8 mm. It crystallized from light petroleum in prisms m.p. 40–41°. (Found: Br, 31.3. $C_{12}H_{15}OBr$ requires Br, 31.4%.)

p-*Bromophenyl* Iso*propyl Ether.*—Prepared conventionally. The *ether* was a fragrant oil, b.p. 119–120°/20 mm., $n_D^{20°}$, 1.5368. It could be crystallized from light petroleum m.p. 17.5°. (Found: C, 51.4; H, 4.2; Br, 36.5. $C_9H_{11}OBr$ requires C, 50.2; H, 5.1; Br, 37.2%.)

p-Cyclo*hexyloxy- and* p-Iso*propyloxybenzoic Acid.*—The above bromides were converted to Grignard reagents by boiling with magnesium and a few drops of ethyl iodide in ether under nitrogen for five hours. The filtered solutions were carbonated in conventional manner with carbon dioxide generated from barium carbonate. The products were isolated conventionally. The *cyclo*hexyl ether (77%) crystallized from ethanol in plates m.p. 176°. (Found: C, 70.7; H, 7.2. $C_{13}H_{16}O_3$ requires C, 70.1; H, 7.3%.) The *iso*propyl ether (72%) crystallized from ethanol in plates m.p. 159° (softens at 140°). (Found: C, 66.1; H, 6.4. $C_{10}H_{12}O_3$ requires C, 66.7; H, 6.7%.)

The acids were dissolved in ether and were treated cautiously with three molecular proportions of lithium methyl and boiled overnight. The acetophenones were isolated, converted to the ketoximes and reduced by sodium amalgam to the amines.

α-^{14}C-labelled *p-cyclo*hexyloxy-α-phenylethylamine was obtained in 40% overall yield (1.28 g.) with an activity of 8,296 counts per minute per mg. α-^{14}C-labelled *p-iso*propyloxy-α-phenylethylamine was obtained in 40% overall yield (0.95 g.) with an activity of 10,562 counts per minute per mg. Yields are calculated on barium carbonate used.

Summary

*p-Cyclo*hexyloxy- and *p-iso*propyloxy-α-phenylethylamine hydrochlorides penetrated with equal facility into rat, rabbit, or monkey cerebral grey matter. A selective localization in rat and rabbit cerebral cortex cannot at present be correlated with analgesic mechanism. There was no localization in peripheral nerve.

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