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DEPLETION OF ADRENAL ASCORBIC ACID AND CHOLESTEROL BY *p-CYCLO*HEXYLOXY-α-PHENYLETHYLAMINE AND RELATED AMINES IN RATS

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Morphine can influence the secretory activity of both adrenal medulla and cortex in experimental animals. It can liberate adrenaline in dogs and cats (Bodo, CoTui, and Benaglia, 1937, 1938; Wada, Tanaka, Hirano, and Taneiti, 1938). It can deplete adrenal ascorbic acid in rats (Nasmyth, 1954), which may indicate pituitary-adrenal activation (Sayers, Sayers, Liang, and Long, 1946). Zauder (1951) also found other evidence for release of ACTH by analgesics. Conversely, the rate of liberation of adrenal hormones may influence analgesic activity. Winter and Flataker (1951) found that ACTH and cortisone antagonized morphine analgesia in rats.

A reduced analgesic response to morphine was shown by dogs (Gross, Holland, Carter, and Christensen, 1948), and by adrenalectomized rats with cortical transplants (Friend and Harris, 1948). The adrenalectomy markedly increased the toxicity of morphine (Torino and Lewis, 1927). Hardy, Wolff, and Goodell (1940) found that adrenaline could reduce the analgesic effect of morphine in man, though Puharich and Goetzl (1947) found that it restored sensitivity to morphine analgesia in adrenalectomized rats.

Section of the nerve supply to the adrenal gland abolishes the release of adrenaline by morphine (Bodo *et al.*, 1938). Release of adrenocortical hormone may follow pituitary activation, since morphine given repeatedly causes adrenal hypertrophy (MacKay and MacKay, 1926; Deansley, 1931; Sung and Way, 1953). The mechanism of pituitary activation is unknown, but the alkaloid can release both adrenaline and histamine, which in turn, under experimental conditions, can release ACTH. Though analgesics appear to concentrate in the adrenals—as Elliot, Chang, Abdou, and Anderson (1949) have found with methadone—it seems unlikely that this engenders the release of hormone, since Vogt (1951) found no increased hormone outflow in dogs' adrenals perfused with morphine. Adrenaline may have a direct effect on the adrenal cortex (Fortier, 1951; Speirs and Sullivan, 1953). *p-Cyclo*hexyloxy-α-phenylethylamine attained a fiftyfold concentration in monkey adrenal glands compared to plasma (Brierley and McCoubrey, 1953), but no difference in concentration between medulla and cortex was found in the rabbit. Though other substances, such as xenon (Featherstone, Steinfield, Gross, and Pittinger, 1952), can concentrate to some extent in adrenal tissue, there is a possibility that such concentration of the amine might reflect a biochemical interaction in the gland which could be related, directly or indirectly, to the analgesic mechanism or to the release of hormones.

It became desirable to determine whether *p-cyclo*hexyloxy-α-phenylethylamine causes pituitary-adrenal activation. The effect of the amine on adrenal ascorbic acid and cholesterol of the rat has been examined for this purpose. For comparison, a number of related amines with different pharmacological properties have been similarly examined.

METHODS

The amine hydrochlorides (20 mg./kg.), or morphine and its derivatives (10 mg./kg.), were given intraperitoneally in aqueous solution at 37° to groups of male albino rats. (When female rats had to be included, comparable groups contained equal numbers of males and females.) The rats were either kept warm overnight in the laboratory before use or were injected in the animal room and left there until killed. Three different strains were used. Strain A was rather less sensitive to analgesics than Strain B. Strain C gave hyperalgesic responses to *p-cyclo*hexyloxy-α-phenylethylamine at 20 mg./kg. and analgesic responses at 30–40 mg./kg. (McCoubrey, 1954). The experiments with different drugs were usually done in parallel, and equal numbers of rats received the different treatments on any one day. They were killed by stunning and bleeding. Adrenal ascorbic acid was estimated by the method of Roe and Kuether (1943), and cholesterol by the method of Sperry and Webb (1950). In one experiment adrenaline was assayed, by the method of Weil-Malherbe and Bone (1952), on adrenal homogenates in 0.1 N-HCl.

The structure and the relevant pharmacology of the phenylethylamines are given by McCoubrey (1954). 2-*n*-Propyldihydromorphine (10 mg./kg.) gave no analgesic response in the rat.

RESULTS

*p-Cyclo*hexyloxy-α-phenylethylamine is referred to as the analgesic *cyclo*hexyl ether, its *N*-allyl derivative as the *N*-allylamine. All the amines examined (except 2-*n*-propyldihydromorphine) markedly depleted ascorbic acid irrespective of pharmacological activity (Table I). The analgesic *cyclo*hexyl ether and its antagonistic *N*-allyl derivative had similar depletory power; the inactive *iso*propyl ether had less activity at the time intervals studied. The hyperalgesic amine had no marked depletory power, which is of interest in view of the hyperalgesic response to morphine seen in rats after treatment with ACTH or cortisone (Winter and Flataker, 1951). Depletion in Strain C at hyper-

TABLE I

DEPLETION OF RAT ADRENAL ASCORBIC ACID AND CHOLESTEROL BY PHENYLETHYLAMINES

Amine hydrochlorides (20 mg./kg.) given intraperitoneally to groups of 4–8 rats. Left adrenals were taken for ascorbic acid (Roe and Kuether, 1943); right for cholesterol (Sperry and Webb, 1950). Comparable groups with saline controls are given. α-Phenylethylamines are designated by their activity, *viz.*, analgesic=*p-cyclo*hexyloxy-α-phenylethylamine; "hyperalgesic"=α-(3-*cyclo*hexyloxy-4-hydroxyphenyl)ethyldimethylamine; inactive=*p-iso*propyloxy-α-phenylethylamine; antagonist=*p-cyclo*hexyloxy-α-phenylethylallylamine. Depletion of ascorbic acid after ACTH is maximal at approx. 60 min. and cholesterol at 3–6 hr. (Long, 1947). The probability of the difference from control being due to chance is indicated as follows: *$P<0.001$, †$P<0.005$, §$P<0.01$, ‖$P<0.02$, other results $P>0.05$.

Drug	Strain of Rats (see text)	Survival (min.)	Ascorbic Acid (mg./100 g.)	Ascorbic Acid % Depletion	Cholesterol (g./100 g.)	Cholesterol % Depletion
Saline	A	30	431±67	—		
Analgesic		30	287±35	33*		
,,		60	273±28	36*		
Inactive		30	311±58	28†		
Saline	A	30	406±28	—		
Analgesic			278±26	32*		
Nalorphine			271±46	33§		
"Hyperalgesic"			305±49	25§		
Antagonist			252±40	38*		
Saline	A	60			2·35± ·49	—
Analgesic					1·74± ·28	26‖
Inactive					2·53± ·73	—
Antagonist					2·73± ·85	—
Saline	A	150	420±49	—	2·30± ·78	—
Analgesic			296±37	30*	2·16± ·45	—
Amphetamine			320±68	24§	2·60± ·56	—
Saline	B	150	593±48	—	3·91± ·46	—
Analgesic			308±25	49*	2·66± ·47	32†
Antagonist			305±43	49*	2·53± ·63	35§
Inactive			409± 8	31*	3·50± ·85	—
Amphetamine			247±43	58*	3·00±1·19	—
Saline	C	150	511±50	—	4·35± ·76	—
Analgesic[1]			278±45	46*	2·65± ·82	39§
Analgesic[2]			231±45	55*	2·22± ·66	50§
2-*n*-Propyldihydromorphine			440±62	12	4·01± ·92	—
Dihydromorphine			340±49	33*	2·36± ·68	46§

[1] Hyperalgesic response after 20 mg./kg. intraperitoneally. [2] Analgesic response after 30 mg./kg. intraperitoneallv.

TABLE II

THE INFLUENCE OF ANALGESIC ANTAGONISTS ON DEPLETION OF RAT ADRENAL ASCORBIC ACID AND CHOLESTEROL BY PHENYLETHYLAMINES

Antagonists (20 mg./kg. i.p.) were given to groups of 6–8 rats before (time in parentheses) the phenylethylamines (20 mg./kg.) or morphine (10 mg./kg.). Survival times are relative to the phenylalkylamines or morphine. The left adrenal was used for ascorbic acid and the right for cholesterol. Analgesic=*p-cyclo*hexyloxy-α-phenylethylamine. Antagonist=*p-cyclo*hexyloxy-α-phenylethylallylamine. Nalorphine=*N*-allylnormorphine.

Strain of Rat (see text)	Pretreatment	Drug	Survival (min.)	Ascorbic acid mg./100 g.±S.D. Saline Control	Ascorbic acid mg./100 g.±S.D. Without Antagonist	Ascorbic acid mg./100 g.±S.D. With Antagonist	Cholesterol g./100 g.±S.D. Saline Control	Cholesterol g./100 g.±S.D. Without Antagonist	Cholesterol g./100 g.±S.D. With Antagonist
A	Nalorphine (5 min.)	Analgesic	30	431±67	261±43	276± 84			
A	,, (5 min.)	Morphine	30	431±67	280±32	342± 66			
A	Antagonist (24 hr.)	Analgesic	30	431±67	287±35	326± 45			
A	,, (24 hr.)	,,	150	420±49	296±37	323± 26	2·30±0·78	2·16±0·45	2·48±0·69
B	,, (24 hr.)	Amphetamine	150	593±48	247±43	370± 35	3·91±0·46	3·00±1·19	3·63±0·54
B	,, (24 hr.)	Analgesic	150	593±48	308±25	369± 38	3·91±0·46	2·66±0·47	1·98±0·61
A	Dibenamine (48 hr.)	,,	150	420±49	296±37	335± 25	2·30±0·78	2·16±0·45	3·10±0·92
C	,, (48 hr.)	,,	150	511±50	221±45	297± 46	4·35±0·76	2·22±0·65	3·62±0·85
				511±35	225±25	289± 15	2·42±0·72	1·35±0·56	1·89±0·38
C	,, (48 hr.)	Antagonist	150	511±35	234±32	322±108	2·42±0·72	1·14±0·34	2·34±0·96

algesic dosage was almost the same as at analgesic dosage.

Long (1947) found that ACTH had no influence on cerebral cortical ascorbic acid content. No depletion occurred with the analgesic *cyclo*hexyl ether, though the drug reached a high concentration in brain (Brierley and McCoubrey, 1953). Eight saline controls gave 38.4 ± 5.6 mg./100 g. of cerebral cortex at 30 min. after injection ; a similar group of rats given the amine had 42.3 ± 6.0 mg./100 g. In the same groups the left adrenal ascorbic acid contents were 406 ± 28 and 278 ± 26 mg./100 g. respectively.

Pretreatment with analgesic antagonists or with dibenamine reduced the depletion of ascorbic acid by analgesics. The effect was not marked (Table II), but was not seen with the combination of analgesic *cyclo*hexyl ether and nalorphine, where antagonism was not well defined. The *N*-allylamine also reduced the depletion by amphetamine, though there was no apparent alteration in the pattern of restlessness and aimless activity due to amphetamine.

Cholesterol was depleted less constantly by these amines. In Strain A this occurred only with the analgesic *cyclo*hexyl ether. In Strain B significant depletion occurred with both this amine and the *N*-allylamine. The inactive *iso*propyl ether had no effect in either strain. In Strain B, but not in Strain A, amphetamine gave inconsistent depletion, which appeared to be reduced by *N*-allylamine pretreatment. The analgesic *cyclo*hexyl ether depleted cholesterol in Strain C at both hyperalgesic and analgesic dosage. Cholesterol and ascorbic acid were reduced after the analgesic dihydromorphine, but its inactive 2-*n*-propyl derivative had little effect on ascorbic acid and none on cholesterol. Dibenamine pretreatment reduced the depletion of cholesterol by the analgesic *cyclo*hexyl ether and the *N*-allylamine, but the *N*-allylamine failed to prevent depletion by the analgesic. The effects of these drugs on cholesterol were small when low cholesterol contents were found in saline controls.

The analgesic amine had previously been found to give no hyperglycaemic response in rats (McCoubrey, 1953), and it seemed unlikely that it liberated adrenaline. Ten rats receiving saline gave an adrenal adrenaline content of $1{,}036 \pm 382$ μg./g. at 30 min. after injection, whereas drug-treated rats had 911 ± 266 μg./g. These values are similar to the control values given by Burn, Hutcheon, and Parker (1950), determined by biological assay after the same method of killing.

Discussion

From the work of Long, Sayers, and their collaborators, depletion of adrenal ascorbic acid or cholesterol is generally accepted as evidence for ACTH release. No basis for ascorbic acid reduction is known, and it may not occur under conditions where it would be expected—for example, in birds (Zarrow and Baldini, 1952). Acetate, a precursor of adrenal cholesterol (Bloch and Rittenberg, 1942), and cholesterol itself, are converted to adrenal steroid hormones by adrenal tissue (Zaffaroni, Hechter, and Pincus, 1951 ; Haines, Nielson, Drake, and Woods, 1951).

Lack of relationship between pharmacological activity and ascorbic acid depletion by these amines is in line with the non-specific nature of this change ; many substances and traumatic conditions reduce adrenal ascorbic acid (Sayers and Sayers, 1949). Analgesics were reasonably consistent in depleting cholesterol, and there seems to be good evidence that they activate the adrenal cortex. Since both the inactive *iso*propyl ether and 2-*n*-propyldihydromorphine failed to lower adrenal cholesterol, this activation might be in some way related to the mechanism of analgesia. Barbiturates, which have some morphine-like properties, though not usually regarded as analgesics (cf. Keats and Beecher, 1950), deplete neither ascorbic acid nor cholesterol (Long, 1947). Though the *N*-allylamine caused cholesterol depletion, this amine is probably a potential analgesic which is self-antagonistic since it can be markedly analgesic. Any relationship between adrenocortical activation and analgesic mechanism must be indirect, since marked lowering of cholesterol occurred when there was no analgesia. Adrenocortical hormones have mainly excitatory effects on brain (for references, see Winter and Flataker, 1951, 1952). Cortisone, like morphine, can be euphoriant, but has no analgesic activity in experimental pain (Lee and Pfeiffer, 1951 ; Vaillancourt, Grokoest, and Ragan, 1951).

The analgesic activity of adrenaline has prompted speculations that morphine analgesia might be mediated in part by adrenomedullary adrenaline (Puharich and Goetzl, 1947). To produce analgesia, a high concentration of adrenaline is probably needed in the cerebral circulation, such as is attained, for example, by intracarotid (Ivy, Goetzl, Harris, and Burrill, 1944) or by intracisternal injection (Leimdorfer and Metzner, 1949). Adrenomedullary adrenaline probably reaches the brain in inadequate concentration for analgesia. Schayer (1951) found no C^{14}-labelled adrenaline in the brain after subcutaneous injection in rats. Uncertain hyperglycaemia after therapeutic doses

of morphine in man (Goodman and Gilman, 1940) suggests that the discharge of adrenaline caused by the alkaloid is not consistent.

It seems preferable to assume as a working hypothesis that adrenocortical activation by analgesics is an incidental effect, in that the mechanism of activation has some resemblance to one stage of the analgesic mechanism. Thus, sudden stress which can release ACTH can also induce analgesia. There seems to be little evidence to relate either pituitary activation or analgesia to release of adrenal adrenaline or noradrenaline. A current hypothesis suggests that ACTH may be released by a neurohumour arising in the hypothalamus (Harris, 1952). Vogt (1954) recently found noradrenaline in the dog's hypothalamus. In experimental animals, the relationship between adrenaline or noradrenaline and analgesia on the one hand, and pituitary activation on the other, is suggestive, in view of the formal chemical similarity between analgesic and sympathomimetic drugs. Dibenamine antagonized the analgesic activity of, and reduced the depletion of cholesterol by, the analgesic *cyclo*hexyl ether. It also reduced the depletion caused by the *N*-allylamine, whereas the latter had no influence on cholesterol depletion by the *cyclo*hexyl ether. It is possible that the analgesic *cyclo*hexyl ether can be antagonized in two different ways.

Summary

1. Four α-phenylethylamines have, irrespective of differences in their pharmacology, approximately equal potency in depleting the adrenal ascorbic acid in rats.

2. The analgesic member of the series was the most consistent in depleting adrenal cholesterol.

3. Previous treatment with analgesic antagonists slightly reduced the depletion of ascorbic acid by analgesics and by amphetamine.

4. Release of adrenaline by the analgesic amine could not be demonstrated, but dibenamine reduced its power to deplete both adrenal ascorbic acid and cholesterol.

5. The results are discussed in relation to the mechanism of analgesia.

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References

Bloch, K., and Rittenberg, D. (1942). *J. biol. Chem.*, **145**, 625.

Bodo, R. C., CoTui, F. W., and Benaglia, A. E. (1937). *J. Pharmacol.*, **61**, 48.

—— —— —— (1938). Ibid., **62**, 88.

Brierley, J. B., and McCoubrey, A. (1953). *Brit. J. Pharmacol.*, **8**, 366.

Burn, J. H., Hutcheon, D. E., and Parker, R. H. O. (1950). Ibid., **5**, 417.

Deansley, R. (1931). *Amer. J. Anat.*, **47**, 475.

Elliot, H. W., Chang, F. N-H., Abdou, Q. A., and Anderson, H. A. (1949). *J. Pharmacol.*, **95**, 494.

Featherstone, R. M., Steinfield, W., Gross, E. G., and Pittinger, C. D. (1952). Ibid., **106**, 468.

Fortier, C. (1951). *Rev. canad. Biol.*, **10**, 454.

Friend, F. J., and Harris, S. C. (1948). *J. Pharmacol.*, **93**, 161.

Goodman, L., and Gilman, A. (1940). *Pharmacological Basis of Therapeutics*, p. 201. N.Y.: MacMillan.

Gross, E. G., Holland, H., Carter, H. R., and Christensen, E. M. (1948). *Anesthesiology*, **9**, 459.

Haines, W. J., Nielson, E. D., Drake, H. A., and Woods, O. R. (1951). *Arch. Biochem.*, **72**, 218.

Hardy, J. D., Wolff, H. G., and Goodell, H. (1940). *J. clin. Invest.*, **19**, 659.

Harris, G. W. (1952). *Ciba Foundation Colloquia on Endocrinology*, **4**, 106. London: Churchill.

Ivy, A. C., Goetzl, F. R., Harris, J. C., and Burrill, D. Y. (1944). *Quart. Bull. Northw. Univ. med. Sch.*, **18**, 298.

Keats, S., and Beecher, H. K. (1950). *J. Pharmacol.*, **100**, 1.

Leimdorfer, A., and Metzner, W. R. T. (1949). *Amer. J. Physiol.*, **157**, 116.

Lee, R. E., and Pfeiffer, C. C. (1951). *Proc. Soc. exp. Biol.*, N.Y., **77**, 752.

Long, C. N. H. (1947). *Recent Progr. Hormone Res.*, **1**, 99.

MacKay, E. M., and MacKay, L. L. (1926). *Proc. Soc. exp. Biol.*, N.Y., **24**, 129.

McCoubrey, A. (1953). *Brit. J. Pharmacol.*, **8**, 22.

—— (1954). Ibid., **9**, 289.

Nasmyth, P. A. (1954). Ibid., **9**, 95.

Puharich, V., and Goetzl, F. R. (1947). *Permanente Fdn. med. Bull.*, **5**, 19.

Roe, J. H., and Kuether, C. A. (1943). *J. biol. Chem.*, **147**, 399.

Sayers, G., and Sayers, M. A. (1949). *Ann. N.Y. Acad. Sci.*, **50**, 522.

—— —— Liang, T., and Long, C. N. H. (1946). *Endocrinology*, **38**, 1.

Schayer, R. W. (1951). *J. biol. Chem.*, **189**, 301.

Speirs, R. S., and Sullivan, J. (1953). *Amer. J. Physiol.*, **173**, 282.

Sperry, W. M., and Webb, M. (1950). *J. biol. Chem.*, **187**, 97.

Sung, C., and Way, E. L. (1953). *J. Pharmacol.*, **108**, 1.

Torino, A., and Lewis, J. T. (1927). *Amer. J. Physiol.*, **81**, 405.

Vaillancourt, G., Grokoest, A. W., and Ragan, C. (1951). *Proc. Soc. exp. Biol., N.Y.*, **78**, 383.

Vogt, M. (1951). *J. Physiol.*, **113**, 129.

—— (1954). Ibid., **123**, 451.

Wada, M., Tanaka, H., Hirano, T., and Taneiti, Y. (1938). *Tohoku J. exp. Med.*, **34**, 52.

Weil-Malherbe, H., and Bone, A. D. (1952). *Biochem. J.*, **51**, 311.

Winter, C. A., and Flataker, L. (1951). *J. Pharmacol.*, **103**, 93.

—— —— (1952). Ibid., **105**, 358.

Zaffaroni, A., Hechter, O., and Pincus, G. (1951). *J. Amer. chem. Soc.*, **73**, 1390.

Zarrow, M. X., and Baldini, J. T. (1952). *Endocrinology*, **50**, 555.

Zauder, H. L. (1951). *J. Pharmacol.*, **101**, 40.