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BRIEF COMMUNICATION

Physiological Disposition of Atropine in the Rat[1]

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HARRISON, S. D., JR., T. R. BOSIN AND R. P. MAICKEL. *Physiological disposition of atropine in the rat.* PHARMAC. BIOCHEM. BEHAV. 2(6) 843–845, 1974. – The physiological disposition of atropine was studied in rats using ^{3}H-labeled drug and a specific assay method. At doses of 1.25 to 10 mg/kg, i.p., the greatest localization was seen in kidney and liver, with tissue: plasma ratios of >10 : 1. Tissue half-lives over the period 0.5 to 4 hr ranged from 40–46 min in plasma to 97–106 min in adipose tissue.

Atropine Tissue levels Physiological disposition

ALTHOUGH pure atropine was first isolated in 1931, and its medical use dates back many centuries, few detailed studies of the physiological disposition of this compound have been published. As mentioned by Evertsbusch and Geiling [3], suitable analytical methodology did not exist until the availability of radiolabeled atropine. These authors used randomly labeled ^{14}C-atropine and followed the time course of radioactivity in various tissues with no attempt to separate metabolites. Albanus *et al.* [1] used ^{3}H-atropine to study the disposition of the drug in mice, with a combination of radioautography and paper chromatography to correct for metabolites. Werner and Schmidt [12] used a combination of ^{3}H- and ^{14}C-atropine and specific procedures in a detailed study of the disposition and metabolism in mice. In addition, Albanus *et al.* [2] used ^{3}H-atropine to study the disposition of total radioactivity in the dog, again ignoring metabolites.

In addition to these studies, a number of reports have appeared describing the metabolic fate of atropine in a variety of animals. As early as 1949, Godeaux and Tonnesen [5] used a bioassay procedure to confirm the metabolism of atropine in rabbit blood and by cat, rabbit and rat liver in situ. Gosselin *et al.* [7] used ^{14}C-atropine to study disposition of radioactivity and excretion of the tropic acid metabolite in mice. Finally, Gabourel and Gosselin [4] examined the urinary excretion of ^{14}C-atropine in mice and rats, and Gosselin *et al.* [6] studied the urinary excretion of ^{14}C-atropine in man. Most recently, Kalser and McLain [9] have examined the excretion of ^{14}C-atropine in man, including studies of the plasma decay curves.

Despite the fact that atropine is widely used in behavioral research, primarily in rats [8,10], no attempts have been made to compare blood or tissue levels of the drug with behavioral effects. Indeed, the physiological disposition of atropine has not been reported in any detail in the rat. The present paper reports on the time course of physiological disposition of ^{3}H-atropine in the rat over a range of doses commonly used in behavioral studies.

METHOD

Adult, male Sprague-Dawley rats (290–350 g) were obtained from Murphy Breeding Laboratories, Plainfield, Indiana and maintained on Purina Lab Chow and tap water ad lib for 7–10 days prior to experimental use. Tritiated atropine (labeled in the para position of the phenyl ring of the tropic acid moiety) was obtained from New England Nuclear Corporation.

Groups of rats were stunned, then decapitated and exsanguinated at various times after i.p. administration of aqueous solutions of various doses of ^{3}H-atropine diluted with unlabeled atropine sulfate so that the dose of radioactivity per rat was 60 μc of ^{3}H. Blood was collected in heparin treated tubes and centrifuged immediately. Tissues were removed immediately; plasma and tissues were stored at −10° C until assay.

Radioactivity was measured in a Packard Tricarb Model

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TABLE 1

TIME COURSE OF LEVELS OF ^{3}H-ATROPINE IN VARIOUS RAT TISSUES

Tissue	Dose (mg/kg, i.p.)	0.05	1	2	4	$t_{1/2}$ min
Plasma	1.25	231± 37 (4)	91± 12 (4)	48± 10 (4)	5.6±1.9 (4)	43
	2.5	430± 78 (3)	163± 37 (3)	93± 12 (3)	11± 5 (3)	46
	5	857± 91 (4)	423± 82 (4)	209± 36 (4)	21± 5 (4)	46
	10	2243 (1)	1015± 96 (4)	375± 71 (3)	63± 9 (3)	40
Brain	1.25	329± 47 (3)	218± 39 (3)	85± 31 (3)	54± 17 (3)	82
	2.5	887± 42 (4)	539± 57 (3)	309± 37 (3)	145± 12 (3)	79
	5	1901± 76 (3)	1226± 109 (3)	794± 26 (3)	296± 20 (3)	71
	10	3639 (1)	2852± 191 (4)	1403±111 (3)	476± 28 (4)	71
Fat	1.25	944±111 (4)	453± 91 (4)	406± 47 (3)	209± 27 (4)	106
	2.5	1983±274 (3)	1645± 194 (3)	974± 93 (3)	511± 78 (3)	97
	5	3742±681 (3)	3444± 601 (3)	2191±278 (3)	1019± 93 (3)	100
	10	8011 (1)	7219± 697 (4)	4667±494 (4)	2312±174 (3)	103
Heart	1.25	601± 93 (3)	257± 33 (3)	102± 12 (3)	39± 13 (3)	53
	2.5	1210± 97 (4)	508± 66 (4)	219± 46 (3)	96± 41 (4)	54
	5	2514±271 (3)	997± 59 (4)	430± 58 (4)	207± 60 (3)	56
	10	5279 (1)	2360± 537 (4)	808±106 (4)	458± 57 (4)	52
Kidney	1.25	2917±168 (3)	1015± 57 (4)	488± 57 (4)	199± 27 (4)	58
	2.5	5612±409 (4)	2404± 360 (3)	995± 93 (4)	411± 78 (4)	56
	5	13206±930 (3)	5880± 397 (4)	1955±216 (4)	822±302 (4)	50
	10	26645 (1)	11681± 944 (4)	3674±378 (3)	1406±271 (4)	53
Liver	1.25	2606± 93 (3)	1178± 111 (4)	718± 76 (3)	346± 39 (4)	74
	2.5	5828±306 (4)	2407± 371 (4)	1631±134 (4)	649± 79 (4)	72
	5	10751±912 (4)	4911± 408 (4)	3079±475 (4)	1607±138 (4)	72
	10	26544 (1)	12988±1012 (4)	6214±612 (4)	3240±319 (4)	76

Each value (ng/g±S.D.) is the mean of values obtained from (N) rats.

4322 liquid scintillation spectrometer, with polyethylene vials and a cocktail consisting of 14 g of 2,5-bis-2-(5-tert-butylbenzoxazolyl)-thiophene and 280 g of naphthalene in a mixture of 2100 ml of toluene and 1400 ml ethylene glycol monomethyl ether [11]. A counting system of 15 ml of this cocktail and 0.5 ml of aqueous solution gives 18% efficiency for ^{3}H with a background of 10 to 15 cpm.

Atropine was determined as follows. Tissues were homogenized in 3 volumes of 0.05 N NCl, using a motor driven Teflon pestle and glass homogenizer. Aliquots of tissue homogenate (1.0 ml) or plasma (1.0 ml) were added to glass stoppered (g.s.) 50 ml shaking tubes containing 25 ml of benzene and 2.0 ml of a saturated aqueous solution of potassium carbonate. After mechanical shaking for 5 min, the tubes were centrifuged. Aliquots (10 ml) of the benzene phase were transferred to g.s. 50 ml shaking tubes containing 2.0 ml of 2.0 N HCl, and the tubes were stoppered, shaken for 5 min, and centrifuged. The benzene phase was removed and discarded. Aliquots (0.5 ml) of the acid phase were then transferred to polyethylene vials containing 15 ml of the liquid scintillation cocktail. The specificity of the method was confirmed by thin-layer chromatography using the system methanol: NH_4OH (100 : 1.5) and silica gel G plates. Under these conditions, only a single radioactive spot was found in the extracts, with an R_f corresponding to authentic atropine. Recovery of authentic atropine was 85–90%.

RESULTS AND DISCUSSION

The results obtained after administration of ^{3}H-atropine in i.p. doses of 1.25, 2.5, 5.0 or 10.0 mg/kg to rats are shown in Table 1. Half-life values were determined from the slopes of the decay curves as estimated by the method of least squares. Several facets are of particular interest. Plasma has the shortest half-life (40–46 min) while adipose tissue (97–106 min) has the longest. Localization is seen in all tissues with tissue: plasma ratios relatively constant over the first 2 hr, then increasing rapidly at 4 hr as plasma levels continue to fall rapidly. Tissue levels decay at a slower rate. In fact, the levels of atropine in tissues appear to have a biphasic decay curve on the basis of selected analyses done at 8 and 12 hr after the larger doses. Plasma values were below the level of detection at all doses after the 4 hr time point.

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