Effects of 2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T) on Radioiodine Distribution in Rats

P. O. Sjödén, T. Archer, and U. Söderberg
Neurophysiological Laboratory, Ulleraker Hospital, Uppsala University S-75017, Uppsala, Sweden

The compounds 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4-dichlorophenoxyacetic acid (2,4-D) have long been used on a world-wide scale as defoliants (GALSTON 1971, WESTING 1971). Several studies have been performed recently in order to investigate the purported toxic and teratogenic action of these compounds (e.g. BÅGE et al. 1973, HANSEN et al. 1971, KHERA and MCKINLEY 1972, WHITEHEAD and PETTIGREW 1972). However, their action on specific biological systems has received only scattered interest (e.g. KOLBERG et al. 1972, SJÖDÉN and SÖDERBERG 1972, 1975, LLOYD et al. 1973). With regard to investigations of thyroid activity, 2,4-D has been shown to cause an elevated uptake of I131 in the thyroid gland (FLORSHEIM and VELCOFF 1962, SÓS and KERTAI 1958). Also, the serum protein bound iodine (PBI) level was decreased after 2,4-D-administration (FLORSHEIM and VELCOFF 1962, FLORSHEIM et al. 1963). This effect was shown not to depend on the pituitary-TSH-thyroid pathway (FLORSHEIM and VELCOFF 1962), but was later suggested by FLORSHEIM et al. (1963) to be caused by a lowering of the thyroxine-binding capacity by serum proteins due to competition by 2,4-D with the binding sites on the protein.

Since no study seems to have been reported on the action of the related compound 2,4,5-T on rat thyroid activity, research on the effects of 2,4,5-T on tissue-uptake of I131, not only in the thyroid gland but in several other tissues as well, must be of definite importance. In the present study, therefore, an effort was made to investigate the effects of 2,4,5-T on I131-distribution, and over a longer period of time than has been done in previous studies (FLORSHEIM and VELCOFF 1962, FLORSHEIM et al. 1963). The focus on radioiodine distribution could be motivated by two arguments. Firstly, the study showing an elevated thyroid uptake of I131 in 2,4-D-treated animals suggests an impaired iodine economy, which could also be a result of administration of 2,4,5-T. Secondly, an associated problem is the role of renal mechanisms in the reported effects. Ethacrynic acid, 2,3-dichloro-4-(2-methylenebutyryl)-phenoxyacetic acid, a related substance, is a potent diuretic agent (DUARTE 1974, EDWARDS et al. 1967) which, at least in bicarbonate-loaded dogs differs from other diuretics by inducing profound chloruresis (EARLEY and FRIEDLER 1964). There remains the possibility that 2,4-D and 2,4,5-T could also have diuretic effects, with an increased renal loss of iodine as a result

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of phenoxyacetic acid-administration. Changes in the serum and tissue/serum radioiodine levels as a result of 2,4,5-T-treatment will be used as indicators of a renal effect.

MATERIALS AND METHODS

EXPERIMENT 1

Forty male Sprague-Dawley rats (AB Anticimex, Sollentuna, Sweden), weighing approximately 400 g at the beginning of the experiment were allowed an acclimation period of 2 weeks at the laboratory, housed in individual cages. Througout the experiment, they were maintained on a low-iodine diet containing between 1.0 and 2.0 µg iodine/g (Astra-Ewos, Södertälje, Sweden) and tap water. The rats were divided into 5 equal groups (N = 8), matched for body weight. All rats in the 4 experimental groups were given an oral injection of 100 mg/kg of 2,4,5-trichlorophenoxyacetic acid (contaminated by less than 1 ppm 2,3,7,8-tetrachlorodibenzo-p-dioxin) in a corn oil suspension (0.4 ml) at different times before killing. Thus, Groups 15, 9, 3, and 1 were given injections of 2,4,5-T, respectively, 15, 9, 3, and 1 days before killing. Group C 3, which served as a control, was given corn oil only, 3 days prior to killing. One day before killing (and immediately after the 2,4,5-T-injection to Group 1) an intramuscular injection of 10 µg of carrier-free I131 in 1 ml isotonic saline was administered in the adductor muscles of the right leg to all groups. One day later, all animals were killed by decapitation, and blood was immediately collected from the body, centrifuged at 4000 rev/min for 8-10 min to yield serum, 0.5 ml of which was used for radioiodine measurement. The following organs were dissected from the bodies: "neck" (the larynx and upper trachea with attached thyroid gland), the whole brain, the left femoral adductor muscles, part of the liver (weight 2 - 3 g), the left kidney, and both testicles. All tissues except the "neck" were immediately weighed to the nearest 0.01 g. Tissues and serum samples were placed in individual plastic tubes in sample containers. The 24-hr tissue-uptake and serum level of I131 were then computed from the analysis in a Packard auto-gamma spectrometer system. Each sample was counted for 20 min. Absolute radioactivity counts were corrected for tissue weight (except for the "neck"), decay, background, and counting time to give counts per minute (CPM) per g or ml of each sample. In addition, tissue/serum (T/S) ratios were computed and, for the "neck", the tissue/muscle (T/M) ratio was included.

EXPERIMENT 2

This experiment was carried out as Exp. 1 with the following exceptions. Sixteen female rats (Wistar) (K.E. Møllegaard, Denmark), weighing approximately 260 g were used. They were divided into 4 equal groups (N = 4), matched for body weight. Groups 3 and 1 were given 100 mg/kg of 2,4,5-T in 0.3 ml of a corn oil suspension, respectively, 3 and 1 days prior to killing. Groups C 3 and C 1

were given identical amounts of oil vehicle on corresponding days.

EXPERIMENT 3

This study deviated from Exp. 1 on the following points. Sixteen male Wistar rats (K. E. Møllegaard, Denmark), weighing around 375 g were used. They were divided into 2 equal groups (N = 8), matched for body weight. All rats were adapted to the oral injection procedure, by giving them 0.4 ml H_2O each day for 8 days prior to the 2,4,5-T-injection. Group 1 was given 100 mg/kg of 2,4,5-T in 0.4 ml oil and Group C 1 was given 0.4 ml of corn oil vehicle 1 day prior to killing.

RESULTS

Radioactivity counts per min (CPM) per ml of serum or g of tissue, T/S and T/M ratios were subjected to analysis of variance according to KIRK (1968). When significant overall differences between groups were found, all paired comparisons were analyzed by the Tukey HSD-test (KIRK 1968, pp. 80-90), using $p < 0.05$ throughout.

The main results have been summarized in Tables I, II, and III, where all figures have been converted to % of control-group values in an attempt to facilitate reading. As is evident from Tables I - III, we obtained essentially the same results in all 3 experiments, indicating that the effects are neither sex- nor strain-dependent. Three types of effects appear: One is a depression of serum radioiodine, most marked on the first day after 2,4,5-T-injection. A second effect is a rise in absolute counts in brain, liver, and female thyroids (Exp. 2) with a peak around Day 3 for brain and female thyroids and Day 1 for liver, despite the fall in serum radioiodine. Consequently, the T/S ratio is markedly increased for all 3 tissues. Finally, of the remaining tissues tested, thyroids and testicles show a moderate or insignificant fall in absolute counts during Day 1, resulting in a rise in the T/S ratios. For muscle and kidney, a moderate Day 1-increase is evident. The fall in serum counts during Day 1 was not reflected by a large enough rise in absolute counts in any organ to explain the fall.

DISCUSSION

The present study shows that a single oral injection of 100 mg/kg of 2,4,5-T depresses 24 hr serum I131 as well as the absolute thyroid counts in male rats, findings that can be most readily explained as effects of an increased renal iodine clearance. Since, at this stage of 2,4,5-T-intoxication, rats show a 20-25 % depression of food- and water-intake (SJÖDÉN et al. unpublished), the effects may partly be caused by a hormonal response to a reduced drinking and partly, or entirely, by an effect on the kidneys resembling the effect of diuretics such as ethacrynic acid, which is also a chlorinated phenoxyacetic acid (DUARTE 1974, EARLEY and FRIEDLER 1964, EDWARDS et al. 1967) and which is known to potentiate chloruresis. Provided that there is no

TABLE I. Time-Response Relations between the Effects of a Single Dose of 2,4,5-T and I^{131}-Distribution in Serum and Neck.[a)]

		Experiment 1			Experiment 2			Experiment 3		
Tissue	Group	Abs. CPM	T/S CPM	T/M CPM	Abs. CPM	T/S CPM	T/M CPM	Abs. CPM	T/S CPM	T/M CPM
Serum	15	103								
	9	106								
	3	88			79					
	1	40*			65*			51*		
Neck	15	101	100	86						
	9	91	91	80						
	3	111	142	93	301*	387*	356*			
	1	91	239*	86	71	127	52	81	139	70

a) Three similar experiments were performed on male Sprague-Dawley (Exp.1), female Wistar (Exp.2), and male Wistar rats (Exp.3). All values are given as percentages of corresponding controls, not given 2,4,5-T.

* = $p < 0.05$.

TABLE II. Time-Response Relations between the Effects of a Single Dose of 2,4,5-T and I^{131}-Distribution in Kidney and Testes.[a)]

		Experiment 1		Experiment 2		Experiment 3	
Tissue	Group	Abs. CPM	T/S CPM	Abs. CPM	T/S CPM	Abs. CPM	T/S CPM
Kidney	15	107	104				
	9	105	102				
	3	110	128	91	116		
	1	67*	175*	161	258*	102	204*
Testes	15	103	97				
	9	103	97				
	3	114	135*				
	1	65*	158*				

a) For legend, see Table I.
* = $p < 0.05$.

TABLE III. Time-Response Relations between the Effects of a Single Dose of 2,4,5-T and I^{131}-Distribution in Brain, Muscle, and Liver.[a)]

		Experiment 1		Experiment 2		Experiment 3	
Tissue	Group	Abs. CPM	T/S CPM	Abs. CPM	T/S CPM	Abs. CPM	T/S CPM
Brain	15	87	83				
	9	93	94				
	3	139*	174	329*	431*		
	1	96	251*	104	170	93	181*
Muscle	15	117	114				
	9	119	117				
	3	117	153	86	111		
	1	104	269*	162*	238*	116	232*
Liver	15	102	98				
	9	117	111				
	3	166*	212	143	183		
	1	143*	378*	197*	323*	199*	394*

a) For legend, see Table I.

* = $p < 0.05$.

shortage of chloride or iodide, both ions are treated equally by the kidney (WILLIAMSON et al. 1962). Therefore, iodine excretion could be similarly enhanced after chlorinated phenoxyacetic acids.

The heavy changes in the pattern of distribution of radioiodine after 2,4,5-T-administration suggest the presence of widespread changes in membrane functions, the most interesting one being the effect on the brain, where iodide can be assumed to be treated like chloride and, therefore, steadily transported out from the cells, mainly as a result of the active transport of sodium. It is worth emphazising that the pump mechanisms responsible for the reabsorption of chloride, iodide and acidic dyes in the kidney are very similar to those pumping material from the part of the brain tissue that is in equilibrium with the cerebrospinal fluid (BÀRÀNY 1973 a,b). However, the physiological role of this system is still largely unknown. Interference of phenoxyacetic acids with membrane pumps have been reported to occur in the skeletal muscle of the rat, where 2,4-D inhibits both the Mg^{++} and the Na^{+} and K^{+} stimulated ATPases (SEILER 1971), an effect that has been suggested to account for the myotonia that is caused by chlorinated phenoxyacetic acids (HEENE 1975).

The observation of signs of marked electrolyte disturbances after 2,4,5-T are of special interest to the understanding of the toxic effects of phenoxyacetic acids on the fetus and the newborn. Thus, substances such as vitamin A, that are known to influence brain pump mechanisms, have also been proved to induce brain malformations during fetal development (KALTER and WARKANY 1961). We have recently shown that electrolyte disturbances, induced by offering lactating rats excess salt, changes the "attitude" of their offspring to salt permanently (FREDRIKSSON et al. 1976). Excess salt-intake by lactating rat dams also has permanent effects on thyroidal parameters related to iodine economy (FREDRIKSSON et al. 1976).

The present findings also provide a new possibility of dose-response measurements of the effect of 2,4,5-T. Preliminary observations (unpublished) have revealed that the effect of 2,4,5-T on iodine distribution is short in that no cumulative effect appears after administration every second day. In previous experiments (FLORSHEIM and VELCOFF 1962, FLORSHEIM et al. 1963, SOS and KERTAI 1958), small doses of 2,4-D have been given repeatedly. Thus, iodine measurements have been performed after a period of prolonged exposure to phenoxyacetic acid. However, the temporal parameters of 2,4,5-T-toxicosis are of greater importance for the effects on I131-distribution than is the total dose given. Thus, repeated doses of 2,4,5-T do not yield effects on I131-distribution which are different from those seen after a single dose (unpublished).

One reason for the present experiment was to investigate the effect of 2,4,5-T on thyroid parameters, since other phenoxyacetic acids such as 2,4-D and a variety of mono-, di-, and tri-iodophenoxyacetic acids have thyroxine

antagonistic activity (KILTGAARD et al. 1951). The present experiment leaves the question of a thyroid effect unanswered, since the thyroidal uptake of iodine is a measure of thyroid activity only when peripheral iodine distribution is uninfluenced. An adequate answer would require measurements of thyroxine and tri-iodothyronine turnover.

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