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BIOCHEMICAL EFFECTS OF FLUOROACETATE ADMINISTRATION IN RAT BRAIN, HEART AND BLOOD

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Abstract—Treatment of rats with fluoroacetate resulted in two phases of behaviour (i) a sedated phase followed by (ii) a tonic extensor convulsive phase. The levels of various labile metabolites such as citric acid, lactic acid, ammonia, free glucose and glycogen, were measured at these two phases in heart, brain and blood. In heart the citric acid level rose in both of the phases, however in brain the level of this metabolite fell at the convulsion following an initial rise during the sedative phase. This alteration in the citrate content of brain could have profound effects on the tricarboxylic acid cycle as a whole and also on the level of compounds, such as GABA, related to the cycle. The implications of such alterations on the overall behavioural patterns are discussed.

FLUOROACETATE is a metabolic inhibitor by virtue of its lethal synthesis to fluorocitrate which competitively inhibits the enzyme aconitase (EC 4.2.1.3. citrate (isocitrate) hydrolyase) thereby blocking the tricarboxylic acid cycle and leading to an accumulation of citric acid in the tissue. Investigations of the effects of fluoroacetate on various tissues have been the subject of many publications.[1–4] There are still many unsolved features concerning the overall effects of this metabolic inhibitor however, and one of these is the mechanism by which fluoroacetate causes convulsions in rats, mice and other animals.[5–7]

MATERIALS AND METHODS

Recrystallised[3] sodium fluoroacetate (British Drug Houses Ltd.) dissolved in 0·9% w/v saline was injected i.p. (250 mg/kg) into adult male Wistar rats (120–150 g). The animals were sacrificed either 20 min after injection of the inhibitor or at the commencement of the convulsion, by immersion in liquid air. The frozen head was severed from the body while a sample of blood was taken from the neck into 10% trichloroacetic acid. The heart was also rapidly removed before it had ceased beating and was immediately transferred to liquid air.

The frozen brain was dissected from the skull, weighed, pulverised in a stainless steel mortar (in the presence of liquid air) and extracted with either 2 volumes of 0·1N HCl for estimating noradrenaline or with 2 volumes of 10% trichloroacetic acid. The frozen hearts were similarly treated. The deproteinised material from the

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trichloroacetic acid extract was centrifuged at 12,000 *g* at 0° after overnight extraction at 2°. The supernatant was neutralised with 3M KOH using methyl orange as external indicator.

Analytical methods. Noradrenaline was extracted and estimated by the method of Shore and Olin.[8] All the other parameters—citrate, pentabromacetone method of Taylor;[9] glycogen, Pfleiderer and Grein;[10] free glucose, Hugget and Nixon;[11] lactic acid, Hohorst[12] and free ammonium ions, Konitzer and Voigt[13]—were estimated in the neutralised trichloroacetic acid extract.

RESULTS

The pattern of behaviour in rats after fluoroacetate treatment was similar to that reported by other workers.[1] There were two quite distinct phases of behaviour. About 10 min after injection of the drug the animals were sedated and akinetic and at this time barbiturate narcosis was potentiated; some 25 min later a tonic extensor convulsion with no prior clonic phase took place, the onset of which was markedly delayed by barbiturate.

The effect of fluoroacetate on the levels of citrate, lactic acid, ammonium ion, free glucose and glycogen in both brain and heart are summarised in Table 1. Fluoroacetate had an effect upon the citrate in both tissues. In heart the level rose eight times in the sedative phase ($P<0{\cdot}001$) and fourteen times at the tonic extensor convulsion when compared to the untreated control. This finding confirms that of Williamson[14] and Bowman[15] who treated a perfused heart preparation with fluoroacetate and found that the citrate level increased by over ten times. The effect of fluoroacetate on the citrate level in brain was however somewhat different. In the sedated phase the citrate level rose to some four times above control values ($P<0{\cdot}01$) but instead of increasing even further at the convulsion, as occurred in the heart, the level fell such that it was only twice that in the control ($P<0{\cdot}02$).

The effect of fluoroacetate on the lactate levels in these two tissues also gave some interesting and unexpected results. In the heart the level of lactate in the sedated phase fell to about one half of that in the control ($P<0{\cdot}001$) but rose to near control levels at the tonic extensor convulsion. By contrast the lactate level in the brain was elevated slightly, although not significantly , in the sedative phase; a significant rise of about 75% ($P<0{\cdot}001$) occurred at the convulsion.

Ammonia was elevated in both heart and brain at the convulsion ($P < 0{\cdot}01$ and $P < 0{\cdot}001$ respectively) but no significant rise could be detected during the sedative phase in either tissue. There was a marked difference in the effect of fluoroacetate and the level of free glucose in these two tissues. In the heart the level of free glucose increased 2-fold in the sedated phase ($P < 0{\cdot}001$) but at the convulsion the level fell to 25 per cent above that of the untreated control. In contrast the level of this sugar in the brain rose in the sedative and convulsive phases by 50 per cent ($P < 0{\cdot}01$) and 75 per cent ($P < 0{\cdot}001$) respectively, *pari passu* with that of lactic acid.

The total glycogen content in both heart and brain was divided into soluble (that which occurred in the trichloroacetic acid extract) and bound (that which occurred in the insoluble residue after trichloroacetic acid extraction). The level of both of these glycogen fractions fell significantly in both heart and brain in the sedative ($P < 0{\cdot}01$) and convulsive phases ($P < 0{\cdot}001$). As a result of these alterations in the level of

TABLE 1. THE EFFECT OF FLUOROACETATE ON LABILE COMPOUNDS IN RAT BRAIN AND HEART

Labile compound	μmoles/kg frozen wt. of tissue					
	Untreated control		Sedated phase		Convulsive phase	
	Brain	Heart	Brain	Heart	Brain	Heart
Citric acid	372·1 ± 25·7*(12)	352·8 ± 28·2 (12)	1246·7 ± 76·9(6)	2874·0 ± 180·9(6)	820·3 ± 59·3(6)	4423·8 ± 242·6(6)
Lactic acid	2363·6 ± 234·1(12)	1697·4 ± 113·6(12)	2901·0 ± 493·1(6)	826·0 ± 115·0(6)	3810·2 ± 552·2(6)	1352·8 ± 135·2(6)
Ammonia	260·5 ± 25·3(12)	418·8 ± 45·1(12)	332·3 ± 43·2(6)	535·7 ± 71·0(6)	472·1 ± 51·2(6)	687·8 ± 73·1(6)
Free glucose	1083·7 ± 53·0(12)	3430·5 ± 224·8(12)	1505·2 ± 107·7(6)	6367·7 ± 523·4(6)	2392·8 ± 252·3(6)	4818·5 ± 280·5(6)
Soluble glycogen	557.5 ± 23·0(12)	2181·6 ± 86·6(12)	388·0 ± 43·0(6)	1150·5 ± 234·4(6)	287·3 ± 35·7(6)	584·0 ± 41·6(6)
Bound glycogen	1543·6 ± 57·8(12)	2918·3 ± 108·3(12)	1066·0 ± 49·4(6)	833·3 ± 238·3(6)	766·7 ± 70·3(6)	416·7 ± 38·0(6)

* Mean ± S.E.M.
Number of animals per group in parenthesis.
Ammonia calculated as $(NH_4)_2SO_4$

glycogen in both tissues it was considered that it might be possible to correlate the level of noradrenaline with this fall. The results of noradrenaline determinations in heart and brain in control, sedative and convulsive phases are shown in Table 2. In neither tissue could any alteration in the noradrenaline concentration be detected in either of the phases after fluoroacetate treatment. It should be pointed out, however, that measuring the absolute level of noradrenaline provides no evidence of an unchanged noradrenaline "turnover".

TABLE 2. THE EFFECT OF FLUOROACETATE ON THE NORADRENALINE CONTENT OF RAT HEART AND BRAIN

	Noradrenaline (μg/g of Tissue)		
	Untreated control	Sedated phase	Convulsive phase
Heart	1·26 ± 0·23*(15)	1·16 ± 0·29 (8)	1·32 ± 0·33 (8)
Brain	0·72 ± 0·16 (16)	0·68 ± 0·22 (8)	0·69 ± 0·32 (8)

* Mean ± S.E.M.
Number of animals per group in parenthesis.

TABLE 3. THE EFFECT OF FLUOROACETATE ON LABILE COMPOUNDS IN RAT BLOOD

Labile compound	μmoles/100 ml of blood		
	Untreated control	Sedated phase	Convulsive phase
Citric acid	6·8 ± 0·3* (12)	34·2 ± 2·8 (6)	55·9 ± 4·8 (6)
Lactic acid	52·5 ± 2·3 (12)	76·6 ± 3·3 (6)	170·8 ± 1·5 (6)
Ammonia	34·8 ± 2·9 (12)	55·2 ± 3·0 (6)	55·6 ± 4·2 (6)
Free glucose	318·9 ± 16·2 (12)	619·0 ± 55·9 (6)	613·3 ± 55·4 (6)

* Mean ± S.E.M.
Number of animals per group in parenthesis
Ammonia calculated as $(NH_4)_2SO_4$

As mentioned previously the effect of fluoroacetate on the levels of various metabolites was studied in blood (Table 3). The citric acid concentration was significantly elevated in both phases after fluoroacetate treatment ($P < 0·001$ in both) as was lactic acid ($P < 0·001$). However the levels of ammonia and free glucose rose in the sedative phase ($P < 0·001$) but further increases did not occur at the convulsion.

DISCUSSION

The findings reported here are consistent with an inhibition of the tricarboxylic acid cycle in both heart and brain during the sedated phase due to lethal synthesis of the fluoroacetate to monofluorocitric acid which inhibits the enzyme aconitase so preventing the conversion of citric acid to *cis*-aconitic acid and thus hindering the oxidation of C-2 fragments via this cycle. In the heart the fall that is seen in lactic acid and the rise in free glucose is difficult to explain on preliminary examination. Citrate in both heart and brain, however, is well known to have an important role in the regulation of glycolysis by virtue of a feedback inhibition of phosphofructokinase

(EC 2.7.1.11. ATP: D-Fructose-6-phosphate 1-phosphotransferase).[15–17] There is little doubt that the elevated citrate level that occurs in heart in the sedated phase would exert a feedback inhibition upon phosphofructokinase so blocking the glycolytic utilization of glucose and causing it to accumulate. However, at this stage the tricarboxylic acid cycle in heart may not be completely blocked. If this is so, because of the inhibition of glucose utilization, lactate will be used as the carbon source to feed into the tricarboxylic acid cycle and will therefore participate in the citrate accumulation. At the convulsion the level of citrate has increased even further in heart indicating a far more complete block of the tricarboxylic acid cycle so preventing the utilization of lactate which now begins to accumulate.

The situation in the brain also raises some interesting problems. In the sedative phase the level of citrate is elevated indicating some inhibition of the tricarboxylic acid cycle; this elevated citrate level will also have feedback effects on phosphofructokinase thus giving rise to the observed elevated glucose level.[18] However, in contrast to cardiac tissue, brain tissue is unable to utilize lactate to any significant extent as an oxidisable substrate[19] and so it will accumulate.

At the convulsion the fall in brain citrate level could possibly be explained by the hypothesis that the citrate level, immediately before the convulsion, rises sufficiently to overcome the fluorocitrate block of aconitase; citrate would thus be oxidised via the tricarboxylic acid cycle. This would result in a decreased citrate level and blockade of aconitase will become re-established.

The consequences of a block in the tricarboxylic acid cycle are numerous, but one of the most important of these will be that the level of gamma-aminobutyric acid (GABA) will rise because its metabolic removal via transamination with α-oxoglutaric acid[20] will be inhibited because of the block in the cycle. It has been shown by Patel and Koenig,[21] who treated cat spinal cord with fluorocitrate, that the level of GABA rose by 82 per cent although no indication was given of the time course of this elevation. Studies on GABA as a possible inhibitory transmitter in the central nervous system have been numerous and although the literature is still conflicting there is still a substantial amount of evidence that a high GABA level will result in a sedated animal.[22–24] Immediately before the convulsion the block of the tricarboxylic acid will be overcome and so the GABA level will fall. There is a considerable volume of evidence in the literature in which measurements of GABA levels have been made at the onset of convulsive seizures and the majority infer that a fall in this compound occurs.[25–27] Further, it has been found that administration of aminooxyacetic acid (AOAA), a compound that causes large increases of GABA in the brain by inhibiting the activity of GABA α-ketoglutarate transaminase,[28] will prevent fluoroacetate convulsive seizures in mice.[29] Preliminary investigations in these laboratories[30] have shown that AOAA administration will prevent fluoroacetate induced convulsive seizures in rats and greatly increases the sedative phase.

Although much of the foregoing is hypothesis experiments are now in progress in which attempts are being made to correlate the levels of citrate and GABA in the brain with the behavioural phases after fluoroacetate treatment in the presence and absence of AOAA.

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