bile, thus reducing the liver content of mercury. The reason for this discrepancy between the results of the present experiments with male rats and those of Alexander and Aaseth [2] with female rats is not clear, but could be due to the sex differences in mercury distribution in this species [7]. The differences in the time of the measurements seems unlikely to be a significant factor, since the increase in the hepatic concentration of mercury at 5 min after treatment with thiol compounds, as reported herein, also was observed at 3 hr (Fig. 1).

At 24 hr, the higher mercury level was still evident in the brain of the rats treated with thiol compounds, whereas the mercury levels of the other organs, plasma and blood did not show any significant difference at this time (Figs. 1 and 2). Magos *et al.* [8] reported that 30 min after cysteine injection the tissue cysteine level increased, and 2 hr after injection it declined nearly to the control level. When the cysteine level recovers to the control level, methylmercury may redistribute according to endogenous thiol levels. As shown in the present results, the effect of the exogenous thiol compounds on the liver, kidney and blood was temporary, but the effect on the brain lasted for up to 24 hr. This also suggests that the brain methylmercury uptake and elimination system is different from those of the other tissues.

In summary, the nature of the methylmercury uptake process in the brain was studied in comparison with that in the other tissues. Stereospecificity was found in the brain methylmercury uptake in the presence of methylmercury cysteine complex. This result may corroborate the assumption that the blood–brain barrier transport system participates in some way in the penetration of methylmercury to the brain.

Acknowledgements—We thank Mr. K. Murao for his assistance in the preparation of the manuscript.

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Biochemical Pharmacology, Vol. 34, No. 11, pp. 2032–2034, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

Effects of the hyperglycaemic agent 3-aminopicolinate on amino acid release by rat muscle *in vitro*

(*Received* 21 *September* 1984; *accepted* 6 *December* 1984)

3-Aminopicolinate is a hyperglycaemic agent *in vivo* that acts, in part, by enhancing gluconeogenesis [1, 2]. Studies to date have not ruled out an additional locus of action in peripheral tissues, and recently it has been suggested that an increase in the release of gluconeogenic precursors from peripheral tissues is the predominant action of the agent [3]. The release of glucogenic amino acids, and alanine in particular, from skeletal muscle is a significant determinant of whole body gluconeogenesis [4, 5]. The involvement of phosphoenolpyruvate carboxykinase in the proposed pathway for the synthesis *de novo* of muscle alanine [6–8] is noteworthy in view of the reported activation of the purified enzyme from rat liver by 3-aminopicolinate [9]. Indeed the hypoglycaemic analogue 3-mercaptopicolinate, an inhibitor of muscle phosphoenolpyruvate carboxykinase [8], diminishes muscle alanine release *in vitro* [6, 8] as well as decreasing hepatic gluconeogenesis through inhibition of the liver enzyme [10]. The effects of 3-aminopicolinate on muscle alanine (and glutamine) release using preparations of rat soleus and extensor digitorum longus (EDL) muscles *in vitro* were therefore investigated.

Materials and methods

Animals. Male Wistar rats, bred in the University of Surrey Animal Unit, were used at a body weight of 70–100 g. They were allowed water *ad libitum* during starvation for 48 hr and were killed at 10.00hr. Soleus and EDL muscles were dissected and incubated for 2 hr, after a 30 min preincubation period, as previously described [11]. Muscles from the contralateral limb of the same animal served as the control in incubations in the absence of 3-aminopicolinate.

Analytical methods. Alanine [6], glutamine [12] and tyrosine [13] were measured in $HClO_4$ extracts of the tissues and incubation media at the end of the incubation period. Phosphoenolpyruvate carboxykinase [14], pyruvate kinase and lactate dehydrogenase [15] were assayed in liver and muscle as described.

Chemicals. Sodium [^{14}C]bicarbonate was obtained from Amersham International (Amersham, Bucks., U.K.). Substrates, enzymes and coenzymes were from Boehringer Co. (London) Ltd. (Lewes, Sussex, U.K.) or Sigma Chemical Co. (Poole, Dorset, U.K.). 3-Aminopicolinic acid (m.p. 209°) was a generous gift from Dr. C. H. Reynolds (The Wellcome Foundation, Dartford, Kent, U.K.) and was prepared fresh and neutralized with NaOH immediately prior to its use in muscle incubations.

Results and discussion

Branched-chain amino acids are the most effective precursors for muscle alanine formation [4, 5]. 3 mM valine stimulated alanine release in incubations of soleus or EDL muscles to similar extents (Table 1). It has been proposed [6–9] that in starved rats valine can serve as a carbon source for alanine formation through its metabolism to citric acid cycle intermediates, and thence to pyruvate via oxaloacetate by the consecutive actions of phosphoenolpyruvate carboxykinase and pyruvate kinase. Transamination of pyruvate with glutamate (formed by transamination of valine) generates alanine via alanine aminotransferase.

Table 1. Effects of 1 mM 3-aminopicolinate (AP) on amino acid release by isolated muscles from 48-hr starved rats

Addition to medium	Amino acid release (μmoles/2 hr per g of tissue)					
	Alanine		Tyrosine		Glutamine	
	−AP	+AP	−AP	+AP	−AP	+AP
M. soleus						
None	0.79 ± 0.07	0.76 ± 0.09	0.36 ± 0.03	0.32 ± 0.02	3.95 ± 0.47	4.56 ± 0.32*
3 mM valine	1.08 ± 0.08	0.97 ± 0.12	0.35 ± 0.02	0.32 ± 0.02	4.53 ± 0.36	6.13 ± 0.52†
M. extensor digitorum longus						
None	0.68 ± 0.06	0.67 ± 0.07	0.34 ± 0.03	0.31 ± 0.03	2.75 ± 0.16	3.80 ± 0.29†
3 mM valine	0.92 ± 0.10	1.02 ± 0.07	0.34 ± 0.02	0.31 ± 0.02	3.58 ± 0.29	5.44 ± 0.28†

Isolated muscles attached to stainless steel frames, were incubated under continuous oxygenation (O_2:CO_2, 95%:5%) in saline–bicarbonate buffer, pH 7.4, for 2 hr with the additions shown. Contralateral muscles from the same animal were incubated in the absence and presence of 1 mM 3-aminopicolinate. Alanine, tyrosine and glutamine were determined in the media at the end of the incubation period. Values are means ± S.E.M. of 12 (alanine, tyrosine) or 8 (glutamine) observations. Statistically significant differences (paired Student's *t*-test) are indicated by: *$P < 0.05$; †$P < 0.01$.

Inhibition of diaphragm or EDL muscle phosphoenolpyruvate carboxykinase by 3-mercaptopicolinate largely abolishes the stimulation of alanine release by valine [6, 8]. In the present study, the hyperglycaemic analogue 3-aminopicolinate had no significant effect on alanine release by soleus or EDL muscle *in vitro*, either in the absence or presence of 3 mM valine (Table 1). Similarly the tissue content of alanine at the end of the incubation period was not affected by 3-aminopicolinate [mean values for all conditions of 0.32 ± 0.04 (16) and 0.36 ± 0.03 (16) μmole/g in soleus and EDL muscle respectively]. In case possible changes in alanine formation *de novo* were being masked by effects of 3-aminopicolinate on amino acid production by proteolysis, tyrosine release (an index of net protein breakdown [13]) was also monitored in these experiments. 3-Aminopicolinate had no effect on tyrosine release by the muscles *in vitro* (Table 1), nor on the tissue content of tyrosine [mean values for all conditions of 0.22 ± 0.02 (16) and 0.21 ± 0.02 (16) μmole/g in soleus and EDL muscle respectively].

In view of the lack of effect of 3-aminopicolinate on muscle alanine formation, the effect of the agent on phosphoenolpyruvate carboxykinase activity was investigated. In agreement with the findings of MacDonald and Lardy [9] with the crude rat liver cytosolic enzyme, no activation of phosphoenolpyruvate carboxykinase was observed with 1 mM 3-aminopicolinate, presumably because activation is already maximal due to the presence of ferroactivator protein. However, we also found no activation with crude extracts of soleus or EDL muscles, which are reported to be devoid of any ferroactivator protein [16]. A 10 min preincubation of liver and muscle extracts with Fe^{2+} and dithiothreitol [9] before assay increased phosphoenolpyruvate carboxykinase activity by about 10%, but the inclusion of 3-aminopicolinate in the pre-incubation mixture caused no further activation of the enzyme. The muscle enzyme may be more like the liver mitochondrial enzyme, which is also insensitive to aminopicolinate activation [16], but further characterization and comparison of muscle phosphoenolpyruvate carboxykinase requires its purification. Phosphoenolpyruvate carboxykinase activity was assayed in the present experiments in the direction of oxaloacetate formation by the $^{14}CO_2$-incorporation method recommended by Duff and Snell (method A, [14]). In assays of liver phosphoenolpyruvate carboxykinase activity in the direction of phosphoenolpyruvate formation (method D, [14]), 1 mM 3-aminopicolinate apparently inhibited enzyme activity by 15% (but see below). The muscle enzyme activities are below the limits of this assay method [14].

Attempts to measure lactate and pyruvate release in muscle incubations in the presence of 3-aminopicolinate were unsuccessful due to interference in the enzymic determinations of these metabolites. Although 3-aminopicolinate contributes to the NADH extinction values measured at 340 nm in the spectrophotometric assays, this "blank" value can be corrected for (for example, in alanine and glutamine determinations). However, lactate and pyruvate determinations both involve the addition of exogenous lactate dehydrogenase and 1 mM 3-aminopicolinate inhibited enzyme activity by 80% in muscle (EDL) and 95% in liver cytosol. Muscle (EDL) cytosol pyruvate kinase activity was also inhibited by 31% by 1 mM 3-aminopicolinate and in an apparently non-competitive manner with respect to phosphoenolpyruvate concentration. However, the assay for pyruvate kinase makes use of exogenous lactate dehydrogenase as a coupling enzyme and the observed inhibitory effects of 3-aminopicolinate may have been secondary to inhibition of the added lactate dehydrogenase. Similarly the apparent inhibition of liver phosphoenolpyruvate carboxykinase activity assayed in the direction of phosphoenolpyruvate formation may have been due to interference with the assay which makes use of added pyruvate kinase and lactate dehydrogenase. Whether the inhibition of lactate dehydrogenase *in vitro* is relevant to the actions of 3-aminopicolinate *in vivo* is difficult to ascertain. However, it is noteworthy that MacDonald *et al.* [2] reported a 3-fold elevation in hepatic lactate concentration, but a negligible change in pyruvate concentration, after 3-aminopicolinate treatment *in vivo*.

Despite the inability of 3-aminopicolinate to activate muscle phosphoenolpyruvate carboxykinase and to affect alanine formation, glutamine release was markedly stimulated by the agent in the presence or absence of valine (Table 1). The valine-promoted incremental increase in glutamine release was stimulated by 171% in soleus and 198% in EDL muscle incubations (Table 1). The pathway by which the carbon of glutamine is derived from valine is not known, but presumably (as with alanine) this involves initial metabolism to citric acid cycle intermediates, but then the transamination of 2-oxoglutarate with valine to form glutamate and further amination to form glutamine. Since alanine formation is not increased by 3-aminopicolinate, the locus of action of the agent on glutamine formation must be at a step(s) beyond 2-oxoglutarate generation. Interestingly, in kidney an effect of 3-aminopicolinate on glutamine metabolism [17–19] has been attributed to a direct stimulation of glutaminase [18]. Regardless of the precise mechanism by which 3-aminopicolinate stimulates muscle glutamine formation, it is

interesting in relation to its hyperglycaemic action that the increased supply of glutamine from muscle is complemented by a stimulation of glutamine uptake and conversion to glucose by the kidney *in vitro* by 3-aminopicolinate [18, 19]. In contrast, Chen and Lardy [3] apparently discount any contribution of renal gluconeogenesis to the hyperglycaemic action of 3-aminopicolinate, since functional renalectomy did not impair the hyperglycaemic response to the agent. In addition to the kidney, the small intestine is a major site of glutamine utilization and the product of this metabolism is alanine [20], which may then serve as a gluconeogenic precursor in the liver. Thus, the increased muscle glutamine production induced by 3-aminopicolinate in the present study could stimulate gluconeogenesis directly in the kidney, or the liver, or indirectly via conversion to alanine by the small intestine.

In summary, the present work has shown that the hyperglycaemic agent, 3-aminopicolinate, has no effect on phosphoenolpyruvate carboxykinase in crude extracts of soleus and EDL muscles. Muscle lactate dehydrogenase was inhibited by the agent and this leads to interference with assay procedures dependent on this enzyme. In muscle incubations with valine, 3-aminopicolinate had no effect on alanine production or on net protein breakdown (tyrosine production), but markedly stimulated glutamine release. The increased glutamine release may serve directly, or indirectly via intestinal conversion to alanine, as a gluconeogenic precursor. Its increased peripheral supply by muscle may contribute to the hyperglycaemic action of the agent *in vivo*.

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Biochemical Pharmacology, Vol. 34, No. 11, pp. 2034–2036, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

β-Adrenergic receptors in rat mammary gland

(*Received* 10 *April* 1984; *accepted* 18 *October* 1984)

It has been shown by several authors that mammary gland responds to catecholamine hormones, assessed by the inhibition of lactose production in guinea pig and mouse mammary slices [1, 2], and that mammary tissue has an adrenaline-responsive adenylate cyclase [3]. It is also known from our studies and those from other laboratories [4–6] that the second messenger cyclic AMP is an important negative regulator of lactogenesis.

Since there are no studies on β-adrenergic receptors in mammary gland, we have attempted to identify and characterize such receptors in the rat mammary secretory tissue with the object of establishing their physiological significance for lactogenesis through regulation of cyclic AMP levels.

In the present work, we reported the results obtained by the use of (−)-[^{3}H]dihydroalprenolol [(−)-[^{3}H]DHA], a potent β-adrenergic antagonist radioligand, to identify, characterize and count β-adrenergic receptors in epithelial cell membranes from lactating rat mammary gland tissue and mammary acini.

Materials and methods

Materials were obtained from the following sources: (−)-[^{3}H]dihydroalprenolol (35.6 Ci/mmole) and [2,8-^{3}H]cyclic AMP (31.5 Ci/mmole) from New England Nuclear, Boston, MA, U.S.A.

All other reagents were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A.

Mammary gland membrane preparation. Membranes were prepared from abdominal mammary glands from lactating Sprague–Dawley rats (9–15 days) by using the method described for heart and other tissues [7]. Protein concentration was determined by the method of Lowry *et al.* [8].

Radioligand binding assay. Binding of (−)-[^{3}H]DHA was determined according to Williams *et al.* [7]. In brief, (−)-[^{3}H]DHA and membrane suspension (0.5 mg protein) were incubated for 15 min with shaking at 37° in 50 mM Tris–HCl buffer (pH 7.5) containing 10 mM $MgCl_2$ to a final volume of 150 μl. The reaction was terminated by dilution to 2 ml with ice-cold incubation buffer. Total binding for