bind to $\alpha_1$-AGP with a high affinity, whereas phenylbutazone affinity is noticeably lower.

Binding of warfarin to $\alpha_1$-AGP is represented in Fig. 1. Displacement of the hyperbola occurs when propranolol is added. The data are analyzed using either a competitive model of inhibition ($K$ values vary with an unchanged $n$ value), or a non-competitive model ($n$ values vary with an unchanged $K$ value). When propranolol is assumed to produce competitive inhibition, the data are adequately fitted (Fig. 1A) whereas the non-competitive pattern of inhibition does not appear to fit the data conveniently (Fig. 1B). The binding parameters of the warfarin, acenocoumarol and phenylbutazone interactions with $\alpha_1$-AGP plus those generally reported for their interactions with HSA [4, 5] were used to simulate binding percentages in serum over a therapeutic range of each drug. Table 2 summarizes the distributions of bound drugs between HSA and $\alpha_1$-AGP in plasma.

*Discussion.* Numerous studies have demonstrated the high-affinity binding of many anionic drugs to HSA [6]. Since this binding to 600 $\mu$M HSA can account for almost all anionic drug binding in plasma, the possibility that other proteins could also bind anionic drugs was poorly investigated.

Most of the anionic drugs studied in this communication are not bound to $\alpha_1$-AGP, or the degree of binding is so low that negligible $\alpha_1$-AGP binding in plasma is then expected. Nevertheless, warfarin and acenocoumarol were found to bind to $\alpha_1$-AGP with a high affinity, even greater than their association constant to HSA. Phenylbutazone is also bound to $\alpha_1$-AGP, but with a lower, intermediate affinity.

Propranolol inhibits the warfarin–$\alpha_1$-AGP binding according to a competitive mechanism. Thus it can be assumed that some acidic and basic drugs share one common site on $\alpha_1$-AGP. As the nature of the binding forces involved has been often questioned, this feature shows that electrostatic interactions are not likely to occur in the high-affinity interactions between these drugs and $\alpha_1$-AGP.

Since we checked the $\alpha_1$-AGP binding of a limited number of acidic drugs, the present work is not intended to ascertain the rules by which anionic drugs are capable of binding to $\alpha_1$-AGP. However, some features are emphasized by this study. Anionic drugs which exhibit a high or intermediate $\alpha_1$-AGP binding affinity do not exhibit any carboxylic moiety, and share a common specific binding site on HSA, called site I by Sudlow *et al.* [7] or the warfarin site by Fehske *et al.* [8] and Sjöholm *et al.* [9]. In contrast, all the drugs poorly or not bound to $\alpha_1$-AGP exhibit carboxylic groups and specifically bind to the other HSA binding site, called site II or the diazepam site respectively by the same authors.

Finally, the simulation of the distribution of bound drugs between $\alpha_1$-AGP and HSA in plasma shows that $\alpha_1$-AGP binding, though relatively low, is not negligible for acenocoumarol and warfarin. Since $\alpha_1$-AGP shows large fluctuations due both to physiological and pathological conditions, the concentration of the drug–$\alpha_1$-AGP combination is likely to vary; the extent of binding in plasma may also change [2].

In summary, the binding of some acidic drugs to $\alpha_1$-AGP was studied by equilibrium dialysis at 37°, pH 7.4. Certain acidic drugs bound to $\alpha_1$-AGP at one binding site with a high affinity. Though the $\alpha_1$-AGP plasma concentration is far lower than the HSA concentration, the association constants of some acidic drugs with $\alpha_1$-AGP are high enough to suggest that binding to $\alpha_1$-AGP will contribute significantly to the total plasma binding of these drugs.

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## Evidence that 3-aminopicolinate stimulates glutamine metabolism by rat renal cortical tissue *in vitro*

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Man *et al.* [1] have recently shown that 3-aminopicolinate, a hyperglycemic agent [2, 3] that stimulates the activity of phosphoenolpyruvate carboxykinase (EC 4.1.1.32) isolated from rat liver [4] and renal cortex [1] accelerates the extraction of glutamine by the rat kidney *in vivo*. However, these authors failed to demonstrate that 3-aminopicolinate affects glutamine removal when rat renal cortical slices are used [1].

The present report shows that suitable concentrations of 3-aminopicolinate exert a stimulatory effect on glutamine

metabolism by isolated rat kidney-cortex tubules. The data obtained strongly suggest that 3-aminopicolinate is an activator of glutaminase (EC 3.5.1.2).

## MATERIALS AND METHODS

*Animals and preparation of isolated kidney tubules.* Kidneys used were from fed male rats (200–300 g) obtained from Iffa-Credo (St Germain-sur-l'Arbresle, France). Kidney-cortex tubules were prepared by collagenase treatment as previously described [5].

*Incubations.* Incubations were carried out at 37° in a shaking water-bath in 25-ml stoppered Erlenmeyer flasks in an atmosphere of 95% $O_2$–5% $CO_2$. Tubules were incubated for 60 min in 4 ml of Krebs–Henseleit buffer (pH 7.40) [6] with 1 or 5 mM L-glutamine both in the presence and the absence of 3-aminopicolinate (sodium salt). The flasks were prepared in duplicate for all experimental conditions. Incubations were terminated by adding perchloric acid (2% final concentration). In all experiments, zero-time flasks were prepared with and without substrate by adding perchloric acid before the tubules. Media were centrifugated for 10 min at 4000 *g* in a refrigerated centrifuge. The supernatant obtained was neutralized with 20% KOH and used for metabolite determinations.

*Analytical methods.* Glutamine, glutamate, aspartate, lactate, pyruvate, 2-oxoglutarate, fumarate, malate, citrate, glucose and ammonia, as well as the dry weight of the tubules added to the flasks, were determined as previously described [5, 7].

*Calculations.* Net substrate utilization and product formation were calculated as the difference between the total flask contents at the start of the experiment and after the period of incubation. The metabolic rates are expressed in μmoles of substances removed or produced per gram dry weight of tubules per hour. The results were analysed by Student's *t*-test for paired data, comparing values obtained in the absence with those obtained in the presence of 3-aminopicolinate.

Complete oxidation of glutamine carbon was calculated as the difference between the amount of glutamine removed and the sum of glutamate, glucose (expressed in $C_3$-units, because two molecules of glutamine are needed for the synthesis of each glucose molecule) and aspartate formed. Flux through glutamate dehydrogenase was calculated as the difference between the flux through glutaminase (taken as the removal of glutamine) and the accumulation of glutamate and aspartate.

*Materials.* L-Glutamine and glutaminase (grade V) were supplied by Sigma Chemical Co. (St. Louis, MO). Other enzymes and coenzymes came from Boehringer Corp. (Meylan, France). The other chemicals were of analytical grade; 3-aminopicolinate was synthesized by the method of Sucharda [8].

## RESULTS AND DISCUSSION

### *Effects of 3-aminopicolinate on the metabolism of 5 mM L-glutamine*

*Effects of low concentrations of 3-aminopicolinate.* The effect of 3-aminopicolinate on 5 mM glutamine metabolism by rat kidney tubules is shown in Table 1. At low concentrations (0.05 and 0.01 mM), 3-aminopicolinate significantly accelerates glutamine removal and its conversion to glutamate, a well-known end-product inhibitor of glutaminase [9], and to ammonia, aspartate and glucose. The fact that glutamine removal is stimulated despite an increased accumulation of glutamate [which occurs despite an increased metabolism of glutamate via glutamate dehydrogenase (EC 1.4.1.2) and aspartate aminotransferase (EC 2.6.1.1)], clearly indicates that 3-aminopicolinate has a stimulatory effect primarily on glutaminase.

Table 1. Effect of 3-aminopicolinate on the metabolism of 5 mM L-glutamine in rat kidney tubules

| Experimental condition | Metabolite removal or production | | | | | Flux through glutamate dehydrogenase | Glutamine completely oxidized |
|---|---|---|---|---|---|---|---|
| | Glutamine | Glutamate | Ammonia | Aspartate | Glucose | | |
| Glutamine (5 mM) | −691.3 ± 30.6 | +147.8 ± 10.9 | +1234.1 ± 69.0 | +1.3 ± 1.3 | +107.7 ± 3.6 | 542.2 | 326.8 |
| Glutamine (5 mM) + 3-aminopicolinate (0.05 mM) | −767.1 ± 37.5** | +173.5 ± 9.8** | +1328.0 ± 53.8* | +10.6 ± 1.9* | +127.7 ± 3.1** | 583.0 | 327.6 |
| Glutamine (5 mM) + 3-aminopicolinate (0.1 mM) | −833.4 ± 47.0** | +199.8 ± 13.7*** | +1433.0 ± 73.1** | +28.0 ± 3.4** | +139.0 ± 5.5** | 605.6 | 327.6 |
| Glutamine (5 mM) + 3-aminopicolinate (0.5 mM) | −722.2 ± 46.0 | +318.7 ± 21.7*** | +1062.5 ± 72.1* | +40.7 ± 7.4** | +54.3 ± 10.8** | 362.8 | 254.2 |
| Glutamine (5 mM) + 3-aminopicolinate (1 mM) | −731.7 ± 45.6 | +336.4 ± 20.5*** | +1057.9 ± 91.0* | +30.9 ± 4.5** | +34.2 ± 7.6*** | 364.4 | 296.0 |

Kidney tubules (16.2 ± 1.3 mg dry wt per flask) were incubated for 60 min as described in Materials and Methods. Results (μmoles/g dry wt) for metabolite removal (−) or production (+) are reported as means ± S.E.M. for six experiments performed in duplicate. Statistical difference was measured by the paired Student's *t*-test against the control without 3-aminopicolinate: *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

Table 2. Effect of 3-aminopicolinate on the metabolism of 1 mM L-glutamine in rat kidney tubules

| Experimental condition | Metabolite removal or production | | | | | Flux through glutamate dehydrogenase | Glutamine completely oxidized |
|---|---|---|---|---|---|---|---|
| | Glutamine | Glutamate | Ammonia | Aspartate | Glucose | | |
| Glutamine (1 mM) | −344.1 ± 37.4 | +53.1 ± 20.0 | +685.3 ± 71.4 | +0.4 ± 1.6 | +67.5 ± 8.2 | 290.6 | 155.6 |
| Glutamine (1 mM) + 3-aminopicolinate (0.05 mM) | −427.2 ± 50.7* | +66.8 ± 3.8** | +857.7 ± 85.4** | +7.6 ± 0.8** | +94.7 ± 12.5* | 352.8 | 163.4 |
| Glutamine (1 mM) + 3-aminopicolinate (0.1 mM) | −467.7 ± 51.6** | +73.4 ± 3.4** | +908.0 ± 94.9** | +14.9 ± 0.5** | +100.8 ± 11.5** | 379.4 | 177.8 |
| Glutamine (1 mM) + 3-aminopicolinate (0.5 mM) | −426.7 ± 49.9** | +152.4 ± 9.4*** | +748.1 ± 76.6* | +20.6 ± 0.7** | +35.4 ± 4.7** | 253.7 | 182.9 |
| Glutamine (1 mM) + 3-aminopicolinate (1.0 mM) | −391.0 ± 49.2* | +163.5 ± 12.4** | +687.6 ± 77.8 | +15.9 ± 0.9** | +12.7 ± 3.1** | 211.6 | 186.2 |
| No added substrate | −6.2 ± 4.2 | −9.0 ± 1.6 | +120.5 ± 19.0 | −9.2 ± 1.2 | +5.1 ± 1.8 | — | — |

Kidney tubules (4.9 ± 0.8 mg dry wt per flask) were incubated for 60 min as described in Materials and Methods. Results (μmoles/g dry wt) for metabolite removal (−) or production (+) are reported as means ± S.E.M. for four experiments performed in duplicate. Statistical difference was measured by the paired Student's *t*-test against the control without 3-aminopicolinate: *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$; the presence of 3-aminopicolinate did not change the data obtained in the absence of added substrate.

The results in Table 1 show that, under these conditions, both the amide nitrogen of glutamine (released by glutaminase) and to a less extent its amino nitrogen (released by glutamate dehydrogenase) contribute to the accelerated synthesis of ammonia.

The accumulation of aspartate in rat kidney tubules found in the presence of 3-aminopicolinate (Table 1), as also the alterations of the metabolite profile observed in rat kidney *in vivo* after its administration [1], provide evidence that 3-aminopicolinate does not cause a stimulation of rat renal phosphoenolpyruvate carboxykinase, as has been shown with the purified enzyme [1], but rather an inhibition. In view of the structural similarity between 3-aminopicolinate quinolinate and 3-mercaptopicolinate [2], two known inhibitors of renal phosphoenolpyruvate carboxykinase that induce an accumulation of aspartate from glutamine in rat renal tissue [10, 11], the suggestion that, in rat kidney tubules, 3-aminopicolinate inhibits the utilisation of oxaloacetate (arising from glutamine) by phosphoenolpyruvate carboxykinase and diverts it to aspartate formation is not surprising. In the presence of 0.5 mM 3-aminopicolinate and with a 5 mM substrate concentration, glucose synthesis is inhibited by 76.8% from L-lactate (N = 4), by 81.9% from pyruvate (N = 4), by 63.9% from glutamate (N = 4), by 58.1% from 2-oxoglutarate (N = 4), by 63.9% from succinate (N = 4), by 77.6% from fumarate (N = 4) and by 68.7% from L-malate (N = 4); a marked inhibition of gluconeogenesis was also observed by Man *et al.* [1] who used lactate, glutamate, 2-oxoglutarate or glutamine as substrates and 3-aminopicolinate at a concentration of 1 mM. A large accumulation of malate also occurs when 2-oxoglutarate, succinate or fumarate are the substrates (data not shown). All these findings are in agreement with an inhibition of phosphoenolpyruvate carboxykinase by 3-aminopicolinate.

Acceleration of glucose synthesis (which paralleled the stimulation of flux through glutamate dehydrogenase; see Table 1), despite inhibition of phosphoenolpyruvate carboxykinase, appears to result from the stimulation by 3-aminopicolinate (0.05 and 0.1 mM) of the entire pathway from glutamine to glucose (secondary to the stimulation of glutaminase).

Thus, our *in vitro* data offer a satisfactory explanation of the *in vivo* observations of Man *et al.* [1] that, under certain conditions, glutamine removal and ammonia formation by the kidney of rats recovering from metabolic acidosis is accelerated by 3-aminopicolinate; failure of these authors to demonstrate such an effect *in vitro* may have been due to the use of renal cortical slices [1], which have been shown to be much less sensitive to experimental maneuvers than isolated kidney tubules when glutamine removal is studied [12].

*Effects of high concentrations of 3-aminopicolinate.* Higher concentrations of 3-aminopicolinate (0.5 and 1 mM) do not affect glutamine removal (Table 1), but greatly alter the metabolic fate of glutamine; glutamate accumulation increases markedly whereas glucose and ammonia formation are inhibited despite an increased accumulation of glutamate (see Table 1). These effects might result from a direct inhibition of glutamate dehydrogenase by 3-aminopicolinate; such an inhibition, which has been demonstrated for quinolinate [13], might explain the large accumulation of glutamate which, in turn, by end-product inhibition, might counteract the stimulation of glutaminase observed with low concentrations (0.05 and 0.1 mM) of 3-aminopicolinate.

Accumulation of glutamate together with inhibition of flux through glutamate dehydrogenase is sufficient to explain the decreased formation of glucose and ammonia caused by high concentrations of 3-aminopicolinate.

*Effects of 3-aminopicolinate on the metabolism of 1 mM L-glutamine*

As shown in Table 2, 3-aminopicolinate affects the metabolism of glutamine, used at a near-physiological concentration, in a way similar to that already observed with a high concentration of glutamine (see Table 1); the main difference is that glutamine utilization and glutaminase are stimulated by 3-aminopicolinate used at concentrations up to 1 mM.

The precise mechanism by which 3-aminopicolinate activates glutaminase remains to be elucidated; however, the fact that 3-aminopicolinate affects phosphoenolpyruvate carboxykinase activity, thanks to its metal-chelating properties [4], suggests, but does not prove, that one or several metals ($Fe^{2+}$, $Mn^{2+}$, $Co^{2+}$) regulate renal glutaminase activity.

In summary, the hyperglycemic agent 3-aminopicolinate used at concentrations of 0.05 and 0.1 mM stimulates the removal of 5 mM glutamine. It also stimulates the accumulation of glutamate and the formation of ammonia, glucose and aspartate by isolated rat kidney-cortex tubules. These effects are consistent with a stimulation of glutaminase and an inhibition of phosphoenolpyruvate carboxykinase by this compound. Higher concentrations (0.5 and 1 mM) of 3-aminopicolinate fail to affect the removal of 5 mM glutamine, but greatly alter the fate of both glutamine carbon and amino nitrogen. Similar effects of 3-aminopicolinate are observed when glutamine is used at a near-physiological concentration.

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