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PURINE SALVAGE PATHWAYS FOR THE BIOSYNTHESIS *IN VITRO* OF ADENINE NUCLEOTIDES IN THE GUINEA PIG VAS DEFERENS*

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Abstract—The guinea pig vas deferens has been found to possess at least two active anabolic pathways for adenine nucleotide biosynthesis. Our studies *in vitro* show that [^{3}H]adenosine and [^{3}H]adenine may be important precursors in purine salvage. Both precursors are eventually converted to [^{3}H]AMP prior to final incorporation into [^{3}H]ATP. [^{3}H]adenosine is transported across the cell membrane and then phosphorylated intracellularly to [^{3}H]AMP with the subsequent formation of [^{3}H]ADP and [^{3}H]ATP. [^{3}H]adenine, on the other hand, is probably converted to [^{3}H]AMP by a mechanism that does not involve [^{3}H]adenosine transport, since 6-nitrobenzylthioguanosine (6-NBTG), a nucleoside transport inhibitor, does not inhibit [^{3}H]adenine utilization. Conversion of [^{3}H]adenosine to [^{3}H]inosine or [^{3}H]hypoxanthine prior to incorporation into [^{3}H]ATP is probably only of minor importance since: (1) 6-NBTG reduces [^{3}H]adenosine conversion to phosphorylated adenine compounds by 90 per cent; and (2) the adenosine deaminase inhibitor, 6-thioguanosine, even at high concentrations could only slightly reduce the amount of [^{3}H]ATP formed from [^{3}H]adenosine. Incubation of vas deferens with either [^{3}H]inosine or [^{3}H]hypoxanthine also failed to result in appreciable labeling of adenine nucleotide pools. The relative contributions of tritium labeled nucleosides and bases to [^{3}H]ATP synthesis *in vitro* in the guinea pig vas deferens are: [^{3}H]adenosine > [^{3}H]adenine ≫ [^{3}H]inosine > [^{3}H]hypoxanthine.

Adenine nucleotide levels in the vas deferens appear to be influenced by a number of pharmacological and surgical treatments. Both reserpine administration and nerve decentralization [1] result in significant increases in endogenous ATP, while 6-OH-dopamine and adrenergic denervation [2] decrease nucleotide concentrations. Rowe *et al.* [3] examined the formation of ATP in guinea pig vas deferens in order to gain some insight into the pathway involved in the biosynthesis of this important nucleotide. Using [^{3}H]adenosine as the sole precursor, they were able to demonstrate formation of [^{3}H]ATP in the vas deferens. In that study, the only radioactive compounds that they reported as being present other than [^{3}H]adenosine and [^{3}H]ATP were [^{3}H]AMP and [^{3}H]ADP.

The present paper is a more detailed examination of the purine salvage pathways involved in ATP synthesis. It was our intention to evaluate the relative importance of adenosine, adenine, inosine and hypoxanthine as possible precursors and/or intermediates of ATP biosynthesis in the guinea pig vas deferens.

METHODS

Male guinea pigs weighing between 300 and 600 g were maintained under a 12-hr light–dark cycle and were given standard laboratory chow and water *ad lib*. Animals were killed by a blow to the head and their vasa deferentia were rapidly removed and the adhering adventitia stripped away. The tissue was blotted dry, weighed and then pre-incubated in 5 ml of Krebs–Henseleit solution of the following composition (mM): CaCl, 113; KCl, 4.8; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 2.5; and glucose, 5.5. This medium was used in order to approximate electrolyte conditions *in vivo*.

Incubation. Incubations were carried out in a Dubnoff metabolic shaker at 37° using 95% oxygen–5% CO_2 as the gaseous phase. When enzyme inhibitors or nucleoside transport blocking agents were used, they were included in the 5-min preincubation period. All reactions were started by the addition of the appropriate radioactive substrate. After incubation the tissue was washed with 50 ml of ice-cold Krebs–Henseleit solution and homogenized with a glass pestle and tube (Duall, size C, Kontes Glass Co) using 5 ml of 0.4 N perchloric acid. This was followed by centrifugation at 9000 *g* for 10 min (Sorvall Superspeed RC2-B). Aliquots from the supernatant solution and from the incubation media were removed for the following procedures: (1) 20 μl for either total tissue (supernatant) or media radioactivity; (2) 10-μl aliquots for analysis of media metabolites; (3) 0.4-ml aliquots of the supernatant solution for neutralization with 0.2 ml of 0.8 N K_2CO_3 followed by centrifugation at 1000 *g* for 5 min to sediment the $KClO_4$ precipitate, and subsequent spotting for chromatographic separation of labeled nucleotides.

Chromatography. Polyethyleneimine ion-exchange thin-layer chromatography was employed for the separation of labeled adenosine and the adenine nucleo-

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tides, AMP, ADP and ATP, as previously described [3]. Descending paper chromatography (Whatman No. 1) or ascending thin-layer Silical gel chromatography was used for the separation of labeled adenosine, adenine, inosine and hypoxanthine from the incubation media. Descending paper chromatography involved cutting a sheet of Whatman No. 1 paper into a 20 cm wide by 60 cm long sheet and spotting 10 μl of a standard aqueous solution containing 1 mg/ml of unlabeled adenosine, inosine, adenine and hypoxanthine at 3- to 4-cm intervals along the origin (which was 10 cm from the top of the sheet). This was followed by spotting 10 μl of sample, drying, and then developing the chromatogram for 20–30 hr at 4° in a solution consisting of butanol, H_2O and NH_4OH (86:14:1). When Silica gel chromatography was employed, 20 × 20 cm sheets were utilized and standards and sample were spotted at the origin which was approximately 1.5 cm from the bottom of the sheet, in a manner similar to the paper chromatograms. The spots were dried and the sheet carefully placed in a chromatography chamber with the solvent (butanol:H_2O:NH_4OH; 86:14:1). The chamber was sealed and the ascending chromatogram run for approximately 10 hr at 4°.

Spots from the various plates were visualized with u.v. light (260 nm), cut out, placed in scintillation vials and eluted with 1 ml of 0.01 N NaOH by shaking for 30 min (120 oscillations/min). Ten ml of counting solution was added and the samples were counted for 10 min using a liquid scintillation counter. Recoveries of labeled adenosine and its phosphorylated derivatives form neutralized acid extracts, or when added directly to the chromatographic plates usually ranged from 90 to 100 per cent. When recoveries were lower, the metabolites were corrected back to 100 per cent. Recovery of labeled adenosine, inosine, adenine and hypoxanthine from chromatography of modified Krebs–Henseleit solution or direct addition to the paper or Silica gel chromatogram was 80–85 per cent.

Media and supernatant-solution radioactivity was determined by addition of the aqueous sample to 10 ml of scintillation counting fluid. Samples from incubation studies consisted of: (1) 20 μl of 0.4 N $HClO_4$ (supernatant from tissue homogenization), (2) 20 μl of modified Krebs–Henseleit media (incubation media) or (3) 1 ml of 0.01 N NaOH (eluate from PEI or paper chromatography sheets). The scintillation fluid consisted of 2,5-diphenyloxazole (PPO, 15 g), 1,4-bis[2-(5-phenyloxazolyl)] benzene (POPOP, 0.9 g) and Biosolv (25 ml for 20-μl aqueous samples or 500 ml for 1-ml aqueous samples) dissolved in 3 l. toluene. Counting was done with a Packard Tri-Carb liquid scintillation spectrometer. Quenching was determined by the use of $[^3H]H_2O$ as an internal standard in vials prepared in a manner similar to the various samples. Counting efficiency was approximately 38 per cent when 1 ml of 0.01 N NaOH was added to the scintillation mixture. An efficiency of 50 per cent was obtained when 20 μl of either 0.4 N $HClO_4$ or modified Krebs–Henseleit media was utilized. All values were corrected for quenching.

Materials. Unlabeled adenine nucleotides, nucleosides and bases were purchased from Sigma Chemical Co., St. Louis, MO. Adenosine[8-^{3}H] (sp. act. 34.2 Ci/m-mole) and adenine[2-^{3}H] (sp. act. 31.7 Ci/m-mole) were purchased from New England Nuclear and inosine[^{3}H] (sp. act. 930 mCi/m-mole) and hypoxanthine[^{3}H-G] (sp. act. 5 Ci/m-mole) were purchased from Amersham/Searle Corp. Polyethyleneimine, ion-exchange chromatographic sheets with fluorescent indicator (green) were purchased from Brinkman Instruments. Whatman No. 1 chromatography papers were purchased from Fisher Scientific Co., and Silica gel chromatograms (6060) with fluorescent indicator (orange) were purchased from Eastman Kodak Co. Biosolv (type III) was purchased from Beckman Instruments. 6-Mercaptopurine was obtained from Burroughs Wellcome Co. 2-Amino-(2-hydroxy-5-nitrobenzyl) thio-9-β-D-ribofuranosylpurine (6-nitrobenzylthioguanosine) was purchased from Raylo Chemicals and 6-thioguanosine was a generous gift from Microbiological Associates, Inc.

RESULTS

Inhibition of [^{3}H]adenosine incorporation into [^{3}H]adenine nucleotides by 6-nitrobenzylthioguanosine (6-NBTG). Inhibition of [^{3}H]adenosine membrane transport should make it possible to determine which pathway – direct phosphorylation or prior metabolism to inosine and/or hypoxanthine – is most important for [^{3}H]ATP synthesis. The adenine nucleoside transport inhibitor, 6-NBTG, was used and the results of these experiments are shown in Fig. 1. The control incorporation of [^{3}H]adenosine into phosphorylated compounds was similar to that reported in previous studies, i.e. although [^{3}H]AMP and [^{3}H]ADP were present in the tissue, [^{3}H]ATP accounted for 80–85 per cent of the total tritiated tissue nucleotides. At a concentration of 4.44×10^{-4} M, 6-NBTG significantly ($P < 0.05$) impaired the formation of all three phosphorylated nucleotides, the total conversion to [^{3}H]nucleotides being reduced by 90 per cent. Inhibition of nucleoside transport with lower concentrations of 6-NBTG (10^{-5}, 10^{-6} M) resulted in similar reductions in accumulation.

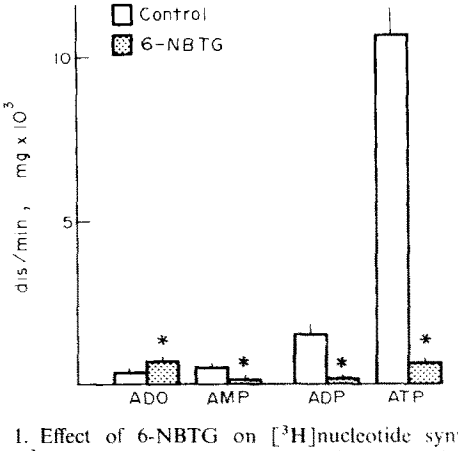

Fig. 1. Effect of 6-NBTG on [^{3}H]nucleotide synthesis from [^{3}H]adenosine (ADO). Guinea pig vasa deferentia were incubated for 15 min with 10 μCi (3×10^{-10} moles/5 ml) of [^{3}H]adenosine both with and without 4.44×10^{-4} M 6-NBTG. Values represent the mean ± S.E.M. of four observations. An asterisk indicates $P < 0.05$.

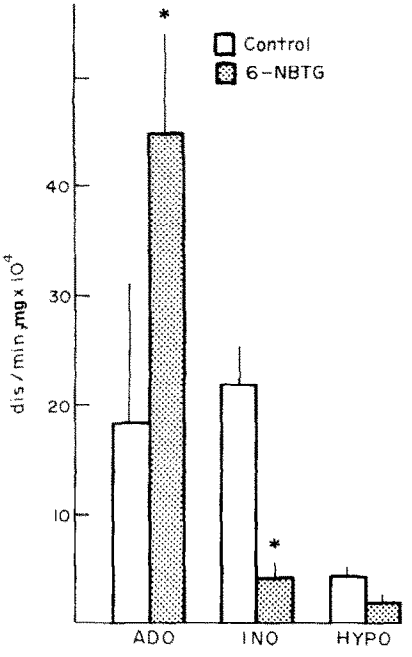

Fig. 2. Effect of 6-NBTG on [^{3}H]inosine (INO) and [^{3}H]hypoxanthine (HYPO) formed from [^{3}H]adenosine (ADO). Guinea pig vasa deferentia were incubated as described in Fig. 1. Values represent the mean ± S.E.M. of four observations. An asterisk indicates $P < 0.05$.

An analysis of radioactivity from the media demonstrated the presence of [^{3}H]inosine and [^{3}H]hypoxanthine in addition to [^{3}H]adenosine (Fig. 2). Apparently both adenosine deaminase and nucleoside phosphorylase are active within the guinea pig vas deferens. [^{3}H]inosine accounted for approximately 49 per cent of the total metabolites. Blockade of adenosine uptake by 6-NBTG significantly increased the amount of [^{3}H]adenosine in the media by 2.5-fold, while reducing [^{3}H]inosine and [^{3}H]hypoxanthine levels ($P < 0.05$).

Effect of adenosine deaminase inhibition on formation of [^{3}H]*nucleotides.* Since 6-NBTG can inhibit the transport of both adenosine and inosine, it was not possible to clearly distinguish which nucleoside was most important as a precursor for ATP synthesis. The experiments shown in Figs. 3 and 4 show the effect of inhibiting adenosine deaminase with 6-thioguanosine (6-ThioG; 1×10^{-3} M) on the formation of [^{3}H]nucleotides and metabolites from [^{3}H]adenosine. Under control conditions, [^{3}H]adenosine accounted for 50 per cent, [^{3}H]inosine for 41 per cent and [^{3}H]hypoxanthine for 10 per cent of the total media radioactivity. The presence of 6-ThioG in the incubation media resulted in an approximate doubling of [^{3}H]adenosine levels, while markedly reducing both [^{3}H]inosine and [^{3}H]hypoxanthine concentrations ($P < 0.05$). The relative proportions of adenosine, inosine and hypoxanthine in the media after adenosine deaminase inhibition were 17:2:1 (86%:10%:5%).

Some inhibition of nucleotide formation in the inhibitor-treated tissue was also apparent. Total [^{3}H]nucleotide accumulation after adenosine deaminase inhibition was reduced by approximately 30 per cent.

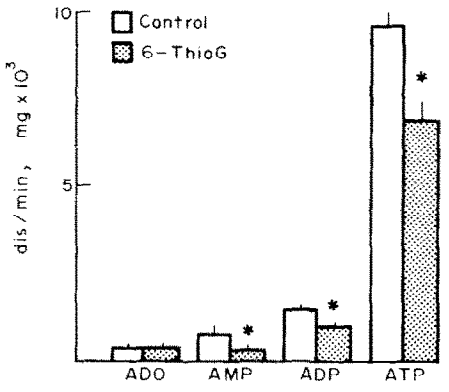

Fig. 3. Effect of 6-ThioG on [^{3}H]nucleotide synthesis from [^{3}H]adenosine (ADO). Guinea pig vasa deferentia were incubated for 15 min with 10 μCi (3×10^{-10} moles/5 ml) of [^{3}H]adenosine both with and without 1×10^{-3} M 6-ThioG. Values represent the mean ± S.E.M. of four observations. An asterisk indicates $P < 0.05$.

Effect of 6-mercaptopurine on [^{3}H]*adenosine incorporation into* [^{3}H]*nucleotides.* It is possible that a portion of the [^{3}H] nucleotide accumulation is due to a sequential metabolism of adenosine to hypoxanthine which could in turn be taken up and incorporated into IMP via hypoxanthine/guanine phosphoribosyltransferase (HPRT) and then eventually to AMP. In order to test this possibility, 6-mercaptopurine, a substrate which competes with hypoxanthine for HPRT, was used to determine its effect on nucleotide synthesis (Fig. 5). Although there appeared to be some tendency toward a reduction in tissue nucleotide concentrations, no significant differences were found between control and drug-treated vasa deferentia. These results at best suggest only a minor role for hypoxanthine as an intermediate in nucleotide biosynthesis from [^{3}H]adenosine.

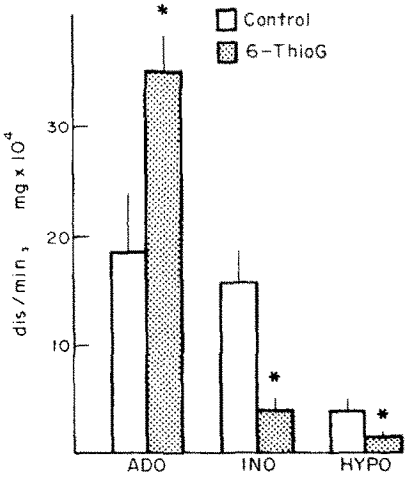

Fig. 4. Effect of 6-ThioG on [^{3}H]inosine (INO) and [^{3}H]hypoxanthine (HYPO) formed from [^{3}H]adenosine (ADO). Guinea pig vasa deferentia were incubated as described in Fig. 3. Values represent the mean ± S.E.M. of four observations. An asterisk indicates $P < 0.05$.

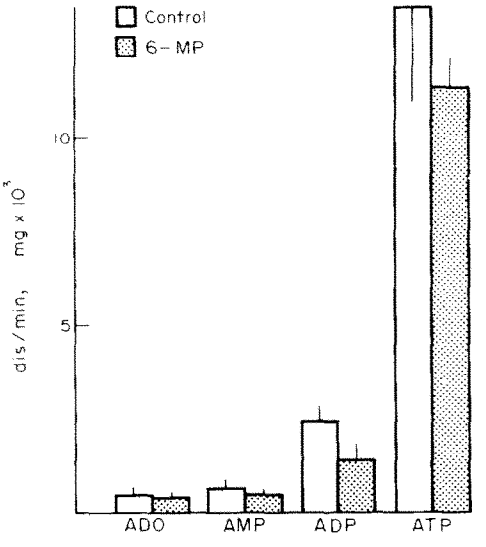

Fig. 5. Effect of 6-mercaptopurine (6-MP) on [^{3}H]nucleotide synthesis from [^{3}H]adenosine (ADO). Guinea pig vasa deferentia were incubated as described in Fig. 1 both with and without 1×10^{-4} M 6-MP. Values represent the mean ± S.E.M. of four observations.

Possible direct precursor role for [^{3}H]*inosine and* [^{3}H]*hypoxanthine in the formation of* [^{3}H]*ATP.* The results reported above indicated that the formation of [^{3}H]ATP can occur through direct phosphorylation of [^{3}H]adenosine and that this seems to be the principle pathway when [^{3}H]adenosine is used as the precursor. Since [^{3}H]inosine and [^{3}H]hypoxanthine formed from [^{3}H]adenosine degradation did not appear to serve as intermediates in ATP synthesis, it was of interest to see if these compounds could directly participate in nucleotide formation.

Comparisons of the relative degrees of incorporation of [^{3}H]adenosine with [^{3}H]inosine and [^{3}H]hypoxanthine into adenine nucleotides are shown in Figs. 6 and 7. Although incubation with [^{3}H]inosine did result in some formation of [^{3}H]AMP, [^{3}H]ADP and [^{3}H]ATP, the amount of incorporation was quite limited (Fig. 6). The total accumulation of phosphorylated compounds in guinea pig vas deferens from [^{3}H]inosine was only 8.6 per cent of that seen when [^{3}H]adenosine was used. The incorporation of [^{3}H]hypoxanthine into [^{3}H]adenine nucleotides was even less extensive than that seen after [^{3}H]inosine (Fig. 7). The amount of incorporation of hypoxanthine into the total nucleotide pool was only 3.7 per cent of that seen for [^{3}H]adenosine. After incubation with either inosine or hypoxanthine, [^{3}H]ATP was the nucleotide present in the highest concentration.

[^{3}H]*Adenine as a precursor for* [^{3}H]*ATP synthesis.* Incubation of guinea pig vasa deferentia with 10μCi [^{3}H]adenosine (1 Ci/m-mole) resulted in a significant amount of [^{3}H]ATP being formed (Fig. 8). [^{3}H]Adenine was incorporated into the [^{3}H]adenine nucleotides in a manner similar to, but to a lesser degree than, [^{3}H]adenosine. The total [^{3}H]nucleotide accumulation from [^{3}H]adenine was 77 per cent of that derived from [^{3}H] adenosine with [^{3}H]ATP being the predominant nucleotide present in the tissue. An analysis of the media radioactivity after incubation showed that over 80 per cent of the label remained in the form of [^{3}H]adenine with only negligible amounts of [^{3}H]adenosine, [^{3}H]inosine and [^{3}H]hypoxanthine being found.

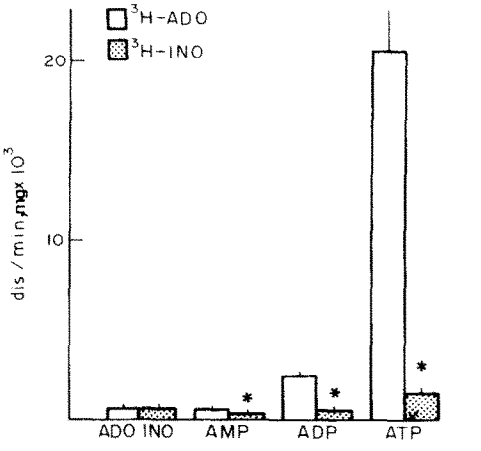

Fig. 6. Synthesis of [^{3}H]adenine nucleotides from either [^{3}H]adenosine (ADO) or [^{3}H]inosine (INO). Guinea pig vasa deferentia were incubated with 10 μCi (1×10^{-8} M) of either [^{3}H]adenosine or [^{3}H]inosine. Values represent the mean ± S.E.M. of four observations. An asterisk indicates $P < 0.05$.

Effect of 6-NBTG on [^{3}H]*adenine incorporation into* [^{3}H]*adenine nucleotides.* A study was made of the effects of nucleoside transport inhibition upon the formation of [^{3}H]ATP from [^{3}H]adenine. This was done in order to determine whether [^{3}H]adenine was being directly incorporated into the ATP pool or was first being converted to [^{3}H]adenosine prior to [^{3}H]ATP synthesis. Incubation with 6-NBTG (4.44×10^{-4} M) had essentially no effect upon the

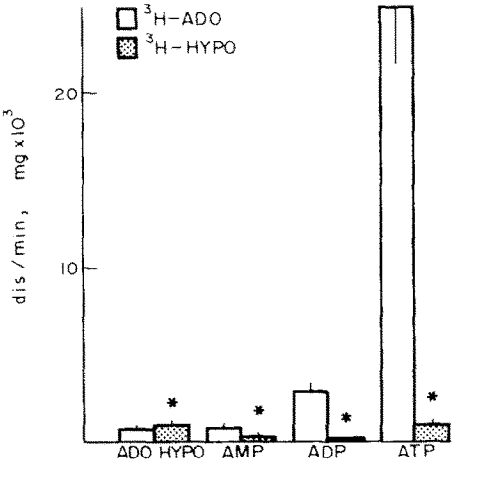

Fig. 7. Synthesis of [^{3}H]adenine nucleotides from either [^{3}H]adenosine (ADO) or [^{3}H]hypoxanthine (HYPO). Guinea pig vasa deferentia were incubated with 10 μCi (1×10^{-8} M) of either [^{3}H]adenosine or [^{3}H]hypoxanthine. Values represent the mean ± S.E.M. of four observations. An asterisk indicates $P < 0.05$.

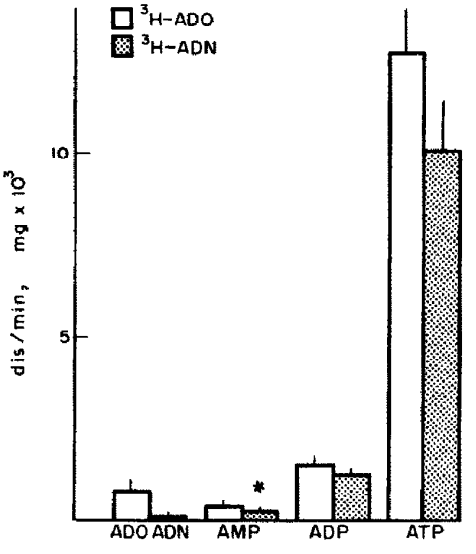

Fig. 8. Synthesis of [^{3}H]adenine nucleotides from either [^{3}H]adenosine (ADO) or [^{3}H]adenine (ADN). Guinea pig vasa deferentia were incubated with 10 μCi (1×10^{-8} M) of either [^{3}H]adenosine or [^{3}H]adenine. Values represent the mean ± S.E.M. of four observations. An asterisk indicates $P < 0.05$.

formation of [^{3}H]ATP (Fig. 9). Although the levels of [^{3}H]AMP were slightly increased ($P < 0.05$), there were no other significant changes in nucleotide formation in the presence of 6-NBTG. These results would seem to rule out the necessity for the conversion of adenine to adenosine prior to its utilization in the synthesis of ATP.

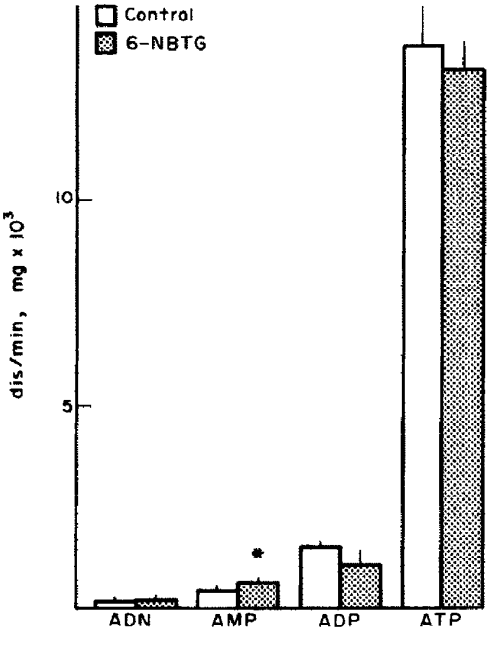

Fig. 9. Effect of 6-NBTG on [^{3}H]nucleotide synthesis from [^{3}H]adenine (ADN). Guinea pig vasa deferentia were incubated for 15 min with 10 μCi (3.2×10^{-10} M) or [^{3}H]adenine both with and without 4.44×10^{-4} M 6-NBTG. Values represent the mean ± S.E.M. of four observations. An asterisk indicates $P < 0.05$.

DISCUSSION

There are three primary pathways for the synthesis of purine nucleotides: (1) synthesis *de novo* of the purine ring from such small molecular weight precursors as glycine, formate, CO_2, glutamine, and aspartic acid [4], (2) condensation of a free purine base with a 5-phosphoribosylpyrophosphate, and (3) phosphorylation of a purine nucleoside. The latter two pathways are included under the general heading of mammalian purine salvage, a process which utilizes or "salvages" preformed purine nucleosides or bases in the synthesis of adenine nucleotides [5]. Purine salvage rather than *de novo* synthesis of adenine nucleotides has been suggested for the maintenance of mammalian tissue nucleotides in most tissues [6–9]. Direct phosphorylation of adenosine to AMP has been suggested as one means whereby adenine nucleotides are replenished in cardiac muscles [7, 10, 11]. Alternative precursors, such as adenine, inosine or hypoxanthine, have also been suggested [12–14].

Recent studies in our laboratory have shown that both the bovine adrenal gland [15] and the guinea pig vas deferens [3] have the ability to incorporate [^{3}H]adenosine into the adenine nucleotide pool, primarily as [^{3}H]ATP. It was evident that adenosine was being incorporated into ATP by the purine salvage pathway, but the specific synthetic reactions were not identified. The present work is a study of the possible salvage pathways and enzymes which may contribute to ATP synthesis in the guinea pig vas deferens.

An analysis of the media after incubation of vas deferens with [^{3}H]adenosine showed that the nucleoside was extensively metabolized to form [^{3}H]inosine and [^{3}H]hypoxanthine. Thus, [^{3}H]ATP biosynthesis could be the result not only of direct phosphorylation of adenosine, but also could proceed through alternative pathways which involved these breakdown products.

The importance of the adenosine phosphorylation pathway was examined indirectly through blockade of adenosine transport by 6-NBTG. This procedure impairs nucleoside phosphorylation, since adenosine transport must either precede or be coincident with phosphorylation. In the guinea pig vas deferens, direct phosphorylation of adenosine appears to be of major importance in the synthesis of ATP, since addition of 6-NBTG to the incubation medium resulted in a 90 per cent decrease in [^{3}H]adenine nucleotide accumulation. In addition, 6-NBTG not only inhibited ATP formation from adenosine, but also reduced the amount of [^{3}H]inosine found in the incubation media. This latter observation supports the possibility that 6-NBTG may inhibit adenosine deaminase in addition to blocking nucleoside transport, since adenosine deaminase is closely associated with nucleoside transport [16]. Such an action has been reported for 6-NBTG in human erythrocytes [16].

[^{3}H]ATP synthesis from [^{3}H]adenosine may also be the result of adenosine conversion to inosine and its subsequent phosphorylation and intraconversion to adenine nucleotides. The ability of inosine to serve

as a precursor was examined in two ways: (1) indirectly by preventing inosine formation through inhibition of adenosine deaminase, and (2) directly by incubating vasa deferentia with [^{3}H]inosine.

Crabtree and Senft [17] have found 6-ThioG (1×10^{-3} M) to be an effective inhibitor of adenosine deaminase *in vitro*. In our studies, inhibition of this enzyme led to a 30 per cent reduction in [^{3}H]ATP formation from [^{3}H]adenosine. As would be expected after deaminase inhibition, media [^{3}H]adenosine levels were increased and inosine levels reduced. When [^{3}H]inosine itself rather than that formed from adenosine breakdown was incubated with vasa deferentia, very little was found to participate in [^{3}H]ATP synthesis. These results suggest that at least 70 per cent of the synthesis *in vitro* of [^{3}H]ATP occurs from direct phosphorylation of adenosine rather than via incorporation of adenosine's deaminated product, inosine. The small degree of inhibition of [^{3}H]ATP synthesis that did occur after deaminase inhibition may actually result from some degree of competitive inhibition of nucleoside transport by 6-ThioG because of the latter compound's close structural similarity to adenosine.

Although inosine is present in interstitial fluid, there is little evidence for direct incorporation via inosine kinase, thus making its purine salvage role questionable.

Wiedmeier *et al.* [14] have recently demonstrated that, when uniformly labeled inosine was perfused through the isolated guinea pig heart, the inosine moiety was first split to hypoxanthine and ribose-1-PO_4 and the base then incorporated into the adenine nucleotides. Thus, inosine may not be directly incorporated into ATP, but may be shunted through hypoxanthine via hypoxanthine/guanine phosphoribosyltransferase (HPRT), an enzyme found in many mammalian tissues. The reaction sequence would involve the conversion of hypoxanthine to IMP by HPRT and then further conversion to AMP. An examination of this pathway failed to demonstrate its importance in the guinea pig vas deferens. Incubation of tissue with [^{3}H]adenosine in the presence of 6-mercaptopurine, a competitive inhibitor of HPRT [18] did not significantly impair [^{3}H]ATP formation. In addition, direct incubation of tissue with [^{3}H]hypoxanthine failed to result in the formation of appreciable quantities of labeled adenine nucleotides including [^{3}H]IMP (unpublished observations). These results strongly suggest that relatively little incorporation of [^{3}H]adenosine into [^{3}H]ATP occurs as the result of sequential metabolism of adenosine into either inosine or hypoxanthine.

The availability of adenine *in vivo* as a potential precursor for ATP synthesis is quite limited [19]. However, small quantities of adenine have been found in urine and the enzyme adenine phosphoribosyltransferase (APRT) which is necessary for direct conversion of adenine to AMP is one of high activity and wide distribution. It was for these reasons that we tested the ability of [^{3}H]adenine to be incorporated into [^{3}H]ATP in the vas deferens. Our results show that adenine can indeed be rapidly incorporated into ATP and to almost the same extent as [^{3}H]adenosine. This incorporation could have occurred along any one of at least three synthetic pathways: (1) conversion of adenine to hypoxanthine via adenine deaminase, (2) conversion of adenine to adenosine via purine nucleoside phosphorylase (PNP) with subsequent direct phosphorylation [20], and (3) conversion of adenine directly to AMP via APRT. The first two pathways have been tentatively ruled out, since adenine deaminase does not appear to be present in mammalian tissues [19] and since blockade of adenosine transport by 6-NBTG did not affect [^{3}H]adenine incorporation. Another explanation for the lack of conversion of adenine to adenosine might involve unfavorable kinetics for this conversion. These observations, therefore, lead us to the conclusion that [^{3}H]adenine is probably incorporated into adenine nucleotides by direct phosphoribosylation and conversion to AMP in the vas deferens. Although this reaction sequence does occur *in vitro*, one should keep in mind that the availability of adenine *in vivo* is relatively limited and, therefore, may not be sufficient to make this a major pathway in the intact animal.

In conclusion, the rates of incorporation *in vitro* of the purine nucleosides and bases, adenosine, inosine, hypoxanthine and adenine, into ATP in the guinea pig vas deferens suggest substantial quantitative differences in the importance of the various potential precursors. Our results show that adenosine and adenine are the predominant precursors in purine salvage with inosine and hypoxanthine playing lesser roles. It appears that the relative contribution of the precursors *in vitro* in the vas deferens is similar to that reported for the perfused rat heart [21] i.e. adenosine > adenine ≫ inosine > hypoxanthine.

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