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# A SITE OF ACTION OF NOVOBIOCIN IN INHIBITING RENAL TUBULAR TRANSPORT OF DRUGS IN THE CHICKEN*

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**Abstract**—Using the systems of renal transport and metabolism, the morphine–morpine-3-ethereal sulfate (MES) and 5-hydroxytryptamine (5HT)–5-hydroxyindole acetic acid (5HIAA) models, a site of action of novobiocin was determined to be on the peritubular side of the renal tubular cell and involved specific inhibition of the organic anion transport system. These conclusions were based on the following observations. Novobiocin blocked the transport of both MES and 5HIAA when these metabolites were administered by intravenous infusion, but novobiocin had no blocking effect when MES and 5HIAA were formed intrarenally from intravenously administered morphine and 5HT respectively. Furthermore, novobiocin had no effect on the transport or metabolism of morphine and 5HT. Tetraethylammonium and catechol transport was not affected, while *para*-aminohippuric acid transport was blocked by novobiocin. The spectrum of blocking activity and site of action of novobiocin were similar but not identical to that of probenecid.

THE PURPOSE of the present investigation was to determine a site of action of novobiocin in blocking the renal transport of drugs. This interest was derived from our earlier study, which showed that novobiocin blocked the biliary excretion of 3-trifluoromethyl-4-nitrophenol (TFM)-glucuronide in rainbow trout exposed to TFM[1] and from the work of others, which indicated that novobiocin can inhibit the hepatic uptake and biliary excretion of bilirubin and other compounds as well as inhibit hepatic glucuronyl transferase.[2–11] By analogy, the question was whether novobiocin would block transport of certain drugs by the renal tubules and, if so, would the site of action be on the luminal or peritubular side of the renal tubular cell? Might metabolism also be inhibited? To investigate the blocking actions of novobiocin, the model systems developed for morphine–morphine ethereal sulfate (MES) and 5-hydroxytryptamine (5HT)–5-hydroxyindole acetic acid (5HIAA)[12–16] were used to establish a site of action of novobiocin on the renal tubular transport system. These two model systems have the advantage of being susceptible to inhibitors which act at different sites within the systems. Thus, the action of inhibitors in blocking the cationic base transport system can be differentiated from the effects of inhibition of intrarenal metabolism of morphine or 5HT. Furthermore, our ability to study renal transport of these metabolites provides yet another possible site of action for differentiating the action of an inhibitor. Because novobiocin has an acid function ($pK_1 = 4{\cdot}3$),[17] one might expect an inhibitory action on the renal anionic transport system. Perhaps the ability of novobiocin to inhibit the metabolism of certain compounds in the liver might be manifested also by an effect on renal metabolism of morphine or 5HT.

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## METHODS

The morphine–morphine ethereal sulfate and serotonin–5-hydroxyindole acetic acid systems for studying renal tubular transport and metabolism were used to study the effects of novobiocin. White Rock laying hens weighing 2·0–3·9 (mean = 2·8) kg were prepared as described in previous publications.[12–16] Plastic tubing with sponge rubber cuffs was sutured on the ureteral orifices for urine collection. The tubing was rinsed continuously at a rate of 0·25 ml/min with distilled water. Urine samples and rinse was collected for 10-min periods; the final volume of each sample was brought up to 10 ml by adding distilled water.

The standard solution for the intravenous infusion (rate of infusion = 0·42 ml/min) into the saphenous vein was made as follows. *p*-Aminohippuric acid (PAH), 16 mg, was dissolved in 200 ml of 0·9% sodium chloride. To this standard solution, one of the following combinations was added: (A) N-[$^{14}CH_3$]morphine hydrochloride (57 mCi/m-mole), 0·3–1 ml stock solution of 10 $\mu$Ci/ml. This final solution gave $^{14}C$ counting ranges of 2623–6335 counts/min/0·1 ml. The [$^{14}C$]morphine was obtained from Amersham/Searle. (B) The same amount of [$^{14}C$]morphine as in A, plus [$^{3}H$]-catechol (ring labeled), 50 mCi/m-mole, from Amersham/Searle. (C) Tetraethyl-[1-$^{14}C$]ammonium (TEA) bromide, 3 mCi/m-mole, infused at 0·009 $\mu$Ci/min (obtained from New England Nuclear Corp.), and [$^{3}H$]catechol. (D) [$^{3}H$]-5-hydroxytryptamine, 6·2 Ci/m-mole, and [$^{14}C$]-5-hydroxyindoleacetic acid, 6·8 mCi/m-mole (New England Nuclear). (E) [$^{3}H$]-5-hydroxytryptamine as in D, plus *N*-methyl-[$^{14}C$]morphine-3-ethereal sulfate (prepared biosynthetically[18]).

The experimental protocol was to infuse the solution for 85–105 min before timed urine collections were started. This period allowed the system to come to a steady state. Then, 10-min control urine samples were collected simultaneously from both kidneys for five such periods (four in one case). After this control period and while the infusion continued, a single dose of novobiocin sodium (50 or 100 mg/kg) dissolved in 0·9% sodium chloride was given intraveneously into a vein in one of the wings. Ten-min urine samples were collected as before; 9–13 periods of collection were made.

Total [$^{14}C$]-radioactivity was determined by placing 0·1 ml of infusion solution or 0·2 ml of diluted urine solution in 15 ml of scintillation fluid prepared with 4 g 2,5-diphenyloxazole (PPO) and 50 mg 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) in a mixture of 1000 ml toluene and 500 ml Triton X-100. Details of the counting pro-procedure were as described previously.[12] PAH was determined colorimetrically in 0·5 ml of the diluted urine sample.[12]

In the chicken, blood from the saphenous vein perfuses the renal portal system of the ipsilateral kidney before it reaches the systemic circulation. The active secretory transport of a substance may be demonstrated by infusing that substance into one saphenous vein. If net secretion occurs, the substance will appear in excess in the urine elaborated by the kidney ipsilateral to the infusion site. If renal metabolism of the drug occurs, the metabolite will also appear in excess in the urine of the infused kidney. The extent to which a particular substance is secreted is quantified by calculating the apparent tubular excretion fraction (ATEF) for that substance. Such quantification for morphine and 5HT as well as for the metabolites of these compounds was performed as described previously,[12, 16] where

$$\frac{EXC_i - EXC_c}{INF} \times 100 = ATEF.$$

TABLE 1. EFFECT OF NOVOBIOCIN ON THE APPARENT TUBULAR EXCRETION FRACTION (ATEF) OF [$^{14}$C]MORPHINE, [$^{3}$H]CATECHOL AND PAH

| Exp. No. | Treatment | Dose (mg/kg) | No. 10-min periods | Apparent tubular excretion fraction | | | Δ From control* | | | [$^{14}$C]MES† |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $^{14}$C | $^{3}$H | PAH | $^{14}$C | $^{3}$H | PAH | % |
| 142 | Control | | 5 | 49·0 ± 3·1 | 53·1 ± 0·1 | 75·1 ± 0·1 | | | | 62 |
| | Novobiocin | 50 | 11 | 48·6 ± 3·6 | 53·8 ± 0·1 | 25·9 ± 0·2 | 0 | 0 | ↓ | 60 |
| 14 | Control | | 4 | 22·9 ± 3·4 | 45·5 ± 6·4 | 60·2 ± 10·3 | | | | 63 |
| | Novobiocin | 100 | 9 | 27·2 ± 1·3 | 54·8 ± 1·7 | 36·5 ± 1·6 | 0 | 0 | ↓ | 62 |
| 146 | Control | | 5 | 26·7 ± 2·2 | 35·8 ± 2·5 | 46·9 ± 0·1 | | | | 71 |
| | Novobiocin | 100 | 9 | 30·7 ± 2·9 | 41·2 ± 2·8 | 30·5 ± 0·1 | 0 | 0 | ↓ | 67 |
| 122 | Control | | 5 | 15·9 ± 0·1 | | 31·9 ± 0·1 | | | | 67 |
| | Novobiocin | 50 | 13 | 30·5 ± 0·1 | | 20·3 ± 0·2 | ↑ | | ↓ | 66 |
| 120 | Control | | 5 | 34·5 ± 3·9 | | 63·2 ± 5·6 | | | | 68 |
| | Novobiocin | 100 | 13 | 54·1 ± 2·8 | | 2·3 ± 2·5 | ↑ | | ↓ | 62 |

* The arrows indicate a significant difference between the control versus novobiocin-treated ATEF values ($P \leq 0.05$) and whether this change was an increase is (↑) or decrease (↓) in ATEF; 0 indicates no significant change.

† Each value is based on an eight-transfer countercurrent distribution analysis of a pooled urine sample from the ipsilateral kidney. The per cent [$^{14}$C]MES is the percentage of the [$^{14}$C]-radioactivity in the sample accountable as [$^{14}$C]MES; the remainder is [$^{14}$C]morphine.

TABLE 2. EFFECT OF NOVOBIOCIN ON THE APPARENT TUBULAR EXCRETION FRACTION (ATEF) OF [$^{14}$C]TETRAETHYLAMMONIUM, [$^{3}$H]CATECHOL AND PAH

| Exp. No. | Treatment | Dose (mg/kg) | No. 10-min periods | Apparent tubular excretion fraction | | | Δ From control* | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $^{14}$C | $^{3}$H | PAH | $^{14}$C | $^{3}$H | PAH |
| 2 | Control | | 5 | 56·2 ± 1·9 | 45·7 ± 1·8 | 61·6 ± 3·0 | | | |
| | Novobiocin | 50 | 10 | 59·5 ± 3·1 | 41·7 ± 2·1 | 31·0 ± 2·2 | 0 | 0 | ↓ |
| 8 | Control | | 5 | 44·0 ± 1·7 | | 41·0 ± 2·0 | | | |
| | Novobiocin | 50 | 11 | 60·1 ± 3·7 | | 21·2 ± 1·8 | ↑ | | ↓ |

* The arrows indicate a significant difference between the control versus novobiocin-treated ATEF values ($P \leq 0{\cdot}05$) and whether this change was an increase (↑) or decrease (↓) in ATEF; 0 indicates no significant change.

TABLE 3. EFFECT OF NOVOBIOCIN ON THE APPARENT TUBULAR EXCRETION FRACTION (ATEF) OF [$^{14}$C]5-HYDROXYINDOLE ACETIC ACID (5HIAA), [$^{3}$H]-5-HYDROXYTRYPTAMINE (5HT) AND PAH

| Exp. No. | Treatment | Dose (mg/kg) | No. 10-min periods | Apparent tubular excretion fraction | | | Δ From control* | | | [$^{3}$H] 5HIAA† |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $^{14}$C | $^{3}$H | PAH | $^{14}$C | $^{3}$H | PAH | % |
| 10 | Control | | 5 | 68·1 ± 4·1 | 44·1 ± 2·4 | 81·7 ± 5·1 | | | | 61 |
| | Novobiocin | 50 | 11 | 13·2 ± 4·3 | 35·6 ± 3·9 | 25·6 ± 5·3 | ↓ | 0 | ↓ | 61 |
| 12 | Control | | 5 | 64·7 ± 3·3 | 40·5 ± 1·9 | 79·5 ± 4·1 | | | | 61 |
| | Novobiocin | 50 | 10 | 26·3 ± 3·9 | 40·8 ± 1·7 | 43·0 ± 3·8 | ↓ | 0 | ↓ | 65 |
| 132 | Control | | 5 | 52·4 ± 3·5 | 27·1 ± 1·8 | 49·2 ± 3·7 | | | | 53 |
| | Novobiocin | 50 | 11 | 11·7 ± 2·5 | 22·7 ± 1·0 | 21·6 ± 2·2 | ↓ | 0 | ↓ | 58 |

* The arrows indicate a significant difference between the control versus novobiocin-treated ATEF values ($P \leq 0.05$) and whether this change was an increase (↑) or decrease (↓) in ATEF; 0 indicates no significant change.

† Each value is based on a nine-transfer countercurrent distribution analysis of a pooled urine sample from three control or three novobiocin administration periods from the ipsilateral kidney. The per cent [$^{3}$H]5HIAA is the percentage of the [$^{3}$H]-radioactivity in the sample accountable as [$^{3}$H]5HIAA; the remainder is [$^{3}$H]5HT.

TABLE 4. EFFECT OF NOVOBIOCIN ON THE APPARENT TUBULAR EXCRETION FRACTION (ATEF) OF [$^{14}$C]MORPHINE-3-ETHEREAL SULFATE (MES), [$^{3}$H]-5-HYDROXYTRYPTAMINE (5HT) AND PAH

| Exp. No. | Treatment | Dose (mg/kg) | No. 10-min periods | Apparent tubular excretion fraction | | | Δ From control* | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $^{14}$C | $^{3}$H | PAH | $^{14}$C | $^{3}$H | PAH |
| 150 | Control | | 5 | 40·5 ± 6·3 | 20·6 ± 1·1 | 34·9 ± 2·5 | | | |
| | Novobiocin | 50 | 11 | 10·5 ± 2·5 | 20·0 ± 1·0 | 10·3 ± 2·0 | ↓ | 0 | ↓ |
| 148 | Control | | 5 | 64·6 ± 4·7 | 34·5 ± 4·7 | 64·5 ± 4·7 | | | |
| | Novobiocin | 50 | 11 | 22·9 ± 2·6 | 27·9 ± 0·9 | 28·2 ± 2·5 | ↓ | 0 | ↓ |
| 134 | Control | | 5 | | 35·3 ± 2·6 | 52·5 ± 3·3 | | | |
| | Novobiocin | 50 | 10 | | 36·0 ± 0·1 | 8·2 ± 2·3 | | 0 | ↓ |

* The arrows indicate a significant difference between the control versus novobiocin-treated ATEF values ($P \leq 0{\cdot}05$) and whether this change was an increase (↑) or decrease (↓) in ATEF; 0 indicates no significant change.

$EXC_I$ was the amount excreted in the urine from the infused side; $EXC_c$ was the amount excreted from the contralateral side, and INF was the amount infused during each 10-min period. An ATEF value greater than 10 per cent is taken as an indication for active transport of that substance. The infusion volume, dilution and sampling parameters chosen were such that ATEF for $^{14}C$ could be calculated where the counts in 0·2 ml of diluted urine and 0·1 ml of [$^{14}C$]-infusion solution could be inserted into the above equation to calculate the ATEF.

In certain experiments where [$^{14}C$]morphine was infused, the total radioactivity in the urine samples from the infused side was fractionated by countercurrent distribution to quantitate that portion excreted as unchanged morphine and MES[12]. In certain other experiments where [$^{3}H$]5HT was used, countercurrent distribution analyses of the ipsilateral urine samples were performed as described previously[16] to quantitate [$^{3}H$]5HT and [$^{3}H$]5HIAA.

## RESULTS

*Effect of novobiocin on PAH, catechol and morphine transport.* As seen in Table 1 during the control periods the ATEF values for PAH, [$^{3}H$]catechol and [$^{14}C$]morphine are high. These values are in agreement with previous results and indicate renal tubular secretion of these compounds.[12–16,18] Note that when novobiocin was administered, the ATEF values for PAH decreased significantly in all experiments. On the other hand, in no case was there a fall in ATEF for morphine or for catechol. Thus, the results indicated that novobiocin blocked PAH transport, but did not block morphine or catechol transport. Countercurrent analyses for morphine indicated there there was no effect of novobiocin on the proportion of $^{14}C$ excreted as [$^{14}C$]-morphine and [$^{14}C$]MES from the ipsilateral infused kidney. In two instances, [$^{14}C$]-ATEF values (bottom, Table 1) were increased, but this effect was not consistently seen and therefore is of doubtful significance. We know from more extensive previous studies that catechol in larger doses than those used in the present experiments does not affect the [$^{14}C$]ATEF of morphine.[13]

*Lack of effect of novobiocin on [$^{14}C$]TEA transport.* Two experiments are shown in Table 2 where [$^{14}C$]TEA was used in place of [$^{14}C$]morphine. In Table 2 the effect of novobiocin in blocking PAH transport is evident as is the lack of effect on [$^{3}H$]-catechol ATEF. In addition, novobiocin did not block TEA transport, since in neither of the two experiments was the [$^{14}C$]TEA–ATEF value decreased.

*Effect of novobiocin on the 5HT–5HIAA system.* The results thus far clearly showed that novobiocin blocked PAH transport without affecting morphine, TEA or catechol transport. Thus, in the experiments shown in Table 3, it was not surprising to see that again the PAH–ATEF values were decreased by novobiocin but that the [$^{3}H$]5HT–ATEF values were not significantly affected. In these experiments, countercurrent analyses of the ipsilateral urine samples showed that the proportion of [$^{3}H$]5HT and [$^{3}H$]5HIAA was not different between control and novobiocin experimental periods. On the other hand, note that the ATEF for [$^{14}C$]5HIAA which was infused was significantly and consistently reduced by novobiocin. Thus, novobiocin blocked the excretion of administered [$^{14}C$]5HIAA but not of [$^{3}H$]5HIAA formed intrarenally from [$^{3}H$]5HT. This type of selective blocking action on 5HIAA is analogous to the selective blocking action of probenecid.[12, 16] Interpreted more broadly, the results indicated that novobiocin blocks the anionic secretory system that handles exogenously

administered PAH or 5HIAA. However, the lack of effect of novobiocin on intracellularly formed 5HIAA places the site of action for novobiocin on the peritubular side of the renal tubular cell. The same interpretation was derived from our next experiment using MES.

*Blocking effect of novobiocin on exogenously administered MES.* In Table 4, novobiocin again reduced PAH ATEF, but had no effect on [$^3$H]5HT–ATEF values. [$^{14}$C]MES, like PAH and exogenously administered 5HIAA, is transported as seen by its high control ATEF values. Administration of novobiocin blocked this transport of [$^{14}$C]MES. This latter result contrasts with the observation in Table 1 where excretion of MES, which was formed intrarenally from morphine, was not affected by novobiocin administration. Thus, the present data again lead to the conclusion that the site of block by novobiocin for the anoinic transport system is on the peritubular side of the renal tubular cell.

## DISCUSSION

It is evident from the results that novobiocin does not block the organic cationic transport system exemplified by TEA, morphine and 5HT. The results unequivocally show that novobiocin does block the organic anionic transport system for PAH, MES and 5HIAA (the latter two, only when administered but not when formed intrarenally). This specificity of novobiocin toward the anionic system is consonant with the independence generally known to exist between the anionic and cationic systems. This selectivity of action of novobiocin closely paralleled that of probenecid on the same transport systems.[12–16, 19, 20] Moreover, we find that novobiocin has no effect on catechol transport, an observation which parallels that made for probenecid by Quebbemann and Rennick.[20] They showed that probenecid had no effect on catechol transport. Thus, the spectrum of blocking action of novobiocin appeared to be similar to that of probenecid.

This similarity goes further, since novobiocin and probenecid block anionic transport at the peritubular side of the renal tubular cell. The arguments for this site of action for probenecid have been developed in previous publications for the morphine and 5HT systems.[12–16] Simply stated, the site of blockade is established by the fact that novobiocin or probenecid does not block excretion of MES or 5HIAA formed within the cell, but does block MES or 5HIAA administered by intravenous infusion. Rennick[19] prefers to put this concept in terms of probenecid being able to differentiate between premetabolic and postmetabolic transport sites.

There is one small but important difference between novobiocin and probenecid. In the present experiments with novobiocin, no effect was found on the ATEF of [$^3$H]5HT. But, previously we had shown that probenecid did possess a slight though significant effect in reducing 5HT transport.[16] Thus, we conclude that in major respects novobiocin and probenecid act in a similar fashion and similar site. However, an observable difference does exist between the two agents.

Finally, in neither the morphine–MES nor the 5HT–5HIAA systems does novobiocin block intrarenal metabolism of the parent compounds. This statement is supported by the evidence that no change occurred in the proportion of parent to metabolite with or without novobiocin treatment. Changes in such proportions have been seen previously with other inhibitors. In the morphine–MES system, catechol inhibited sulfate conjugation of [$^{14}$C]morphine without affecting [$^{14}$C]ATEF values.[13]

In the 5HT–5HIAA system, the monoamine oxidase inhibitor, pheniprazine, blocked the oxidation of 5HT to 5HIAA within the renal tubular cell with no effect on the amount of 5HT crossing the peritubular membrane into the cell. This lack of effect of novobiocin on metabolism of morphine and 5HT is of interest, since in the liver novobiocin has been shown to inhibit glucuronyl transferase.[1–7] Thus, in the present work, we were able to show an effect of novobiocin in blocking transport in the absence of a possibly complicating effect on metabolism.

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