Urological Research 8, 101-106 (1980)

Urological Research

Permeability of Heterotopic and Homotopic Rat Urinary Bladders to Water, Sodium and a Carcinogenic Aromatic Amine

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Accepted: September 17, 1979

Summary. A heterotopic bladder model has been described previously for the study of urinary bladder carcinogenesis and the cocarcinogenic role of urine. In the studies presented here, the permeability coefficients of heterotopic and homotopic bladders to water, sodium, and a carcinogen, 2-acetylaminofluorene (AAF), were measured. There were no significant differences in the sodium and AAF permeabilities, but the water permeability coefficient was significantly increased (24%) in the heterotopic bladder. However, when translated into percent absorption per hour there was no difference between the two groups because the larger volume of the heterotopic bladder cancels out the effect of the increased permeability coefficient.

The heterotopic bladder epithelium maintains its functional status as measured by these permeability studies, and therefore the heterotopic bladder is a satisfactory model for the study of bladder carcinogenesis.

Key words: Heterotopic bladder - Bladder permeability - Permeability coefficient - Sodium permeability - Water permeability - AAF permeability.

A heterotopic urinary bladder model has been developed as a working model to study the role of urine in urinary bladder carcinogenesis by chemical carcinogens. The heterotopic urinary bladder was prepared by connecting an Ommaya reservoir (Heyer-Schulte Corp., Goleta, Calif.) to the neck of a freshly removed bladder and transplanting it to the back of a syngeneic rat (13). The mucosa of the transplant underwent ischaemic necrosis initially but re-epithelialization occurred within 2 weeks as a result of regeneration probably originating from the trigone or the distal portion of the ureters. The regenerated epithelium consisted of a three cell thick epithelium indistinguishable from the normal one (13).

To be a satisfactory model of a urine-free bladder, the heterotopic bladder must approximate the normal bladder in its functional characteristics as well as its morphology. One of the important functions of the urinary bladder is its resistance to permeation by electrolytes and small hydrophilic molecules. Morphologically, this unique function is attributed to 3 structural elements of the plasma membrane of superficial cells, namely, asymmetrical unit membrane plaque and interplaque membrane at the luminal surface and tight junctions at the lateral surfaces (2, 5-8, 10-12).

The purpose of the study reported here is to measure and compare the permeability of the heterotopic and homotopic urinary bladder to water, sodium, and a carcinogenic aromatic amine, 2-acetylaminofluorene (AAF).

MATERIAL AND METHODS

General

The test compounds used are radioactive and all solutions used were isotonic (0.9% saline). They were warmed to 37°C prior to injection and bladders were filled to a pressure of 5-6 cm of water.

Initially, two different time periods (1 and 4 hours) were used to check the reproducibility of the system since the permeability coefficient should be independent of time. Subsequently other time intervals (0.25, 0.50, 2 and 6 hours) were studied to clarify the effects of time in this experimental design. Also a limited series of animals was studied in a ten-fold reduced specific

0300-5623/80/0008/0101/$ 01.20

activity level of isotopes to determine whether the specific activity introduced any errors in the system. Water and AAF permeability were studied simultaneously. Sodium was studied separately due to an overlap in energy emission levels.

Preparation of Stock Solutions

Tritiated Water - AAF. One hundred μCi of 9-^{14}C-2 acetylaminofluorene (AAF) with a specific activity of 13.17 mCi/mM was dissolved in 0.1 ml of 95% ethanol. To this 100 μCi of tritiated water (THO) with a specific activity of 250 μCi/g was added. The solution was then brought to 50 ml with 0.9% saline to give a final activity of 2 μCi THO and 2 μCi AAF per ml.

Sodium - 22. For the homotopic bladder studies, 100 μCi of sodium-22 sodium chloride in water (1 mCi/ml) was diluted to 2 ml with 0.9% saline to give a specific activity of 50 μCi/ml. A 1:100 dilution was made for internal standardization.

For the heterotopic bladder studies, 100 μCi of sodium-22 was diluted to 6 ml with 0.9% saline to give a specific activity of 16.7 μCi/ml. The increased volume was needed to fill the reservoir in addition to the heterotopic bladder. A 1:100 dilution of this stock solution was made for internal standardization.

Preparation of Animals. Heterotopic bladder systems were prepared in 100-150 g Fisher female rats (Charles River Breeding Co., Wilmington, Mass.) as previously described (13) except that the donor animals received 5 mg tobramycin intramuscularly one day prior to the bladder transplantation (2). Control animals were housed under similar conditions as the animals with heterotopic bladders. Permeability tests were performed at four weeks following transplantation because by this time, regeneration of the epithelium had been completed, and the superficial cells had regained the ultrastructural cell surface characteristics (unpublished data).

Heterotopic Bladder Permeability to Water and AAF. Animals were anaesthetised with ketamine hydrochloride 100 mg/kg intramuscularly. The hair over the heterotopic bladder and on the lower abdominal wall was shaved. A lower midline abdominal incision was made, and the ureters were intubated with heat-drawn polyethylene catheters (P. E. 150, Clay Adams, Parsippany, N. J.) through ureterotomies. The animals were placed in the left lateral position and the skin over the heterotopic bladder was prepared with povidone-iodine solution. The connector between the reservoir and the heterotopic bladder was exposed through a small incision. The contents of the reservoir system were aspirated percutaneously and cultured on brain heart infusion plates. The system was irrigated with 0.3 ml of 0.9% saline and aspirated again.

The reservoir system was filled with doubly labeled isotonic stock solution to a pressure of 5 to 6 cm of water as measured by manometer. The reservoir was pumped gently several times to insure homogeneity of the contents of the system and then the connector was clamped. At the termination of the period of study, the heterotopic bladder and reservoir were excised intact, still leaving the connector clamped. The bladder was cut away from the system into a vial of 5 ml of 0.9% saline, bisected and rinsed. It was placed in a vial with 1 ml of Soluene-100 (Packard) and kept in a 37°C oven overnight. The reservoir was emptied into 5 ml of 0.9% saline and washed by irrigating it several times with 0.9% saline. Since AAF is soluble in alcohol, five washes of 0.5 ml each of 95% ethanol were performed.

The 5 ml initial irrigation of the reservoir was diluted to 10 ml with 0.9% saline in a volumetric flask. A 0.5 ml aliquot was taken for liquid scintillation counting after adding 10 ml of Dimilume-30 (Packard Instrument Co., Inc., Downers Grove, Ill.) to the vial. Each alcohol wash was also counted (Packard Tricarb Liquid Scintillation Spectrometer Model 3385). The activity of each isotope in the reservoir was summed.

The 5 ml bladder washing was diluted to 10 ml with 0.9% saline in a volumetric flask and a 0.5 ml aliquot was taken for liquid scintillation counting. The dissolved bladder tissue was divided into two equal aliquots (approximately 0.5 ml each) and counted. The activity of each isotope in the bladder tissue and bladder wash was summed.

Aliquots of 0.05 ml of the stock solution were added to .45 ml of 0.9% saline and counted to calculate the total amount of isotopes infused into the system.

The amount of tritiated water (THO) in the heterotopic bladder at the start of the study was calculated by subtracting the amount of THO recovered from the reservoir from the total dose instilled in the system. Bladder volume was calculated from these figures.

Because AAF is in suspension in 0.9% saline, the ratio of AAF to THO can vary. Since the fluid in the bladder and the reservoir were mixed prior to clamping the connector, the ratio of AAF to THO in the reservoir was used to calculate the initial amount of AAF in the Bladder.

Knowing the amount of isotopes in the bladder initially and at the end of the study, the volume of the bladder, and the duration of the study, the permeability coefficient (P) for water (THO) and AAF was calculated using Fick's law as described by Fellows and Marshall (3).

$$\frac{N_t}{N_o} = e^{-kt} \quad \text{and} \quad P = \frac{kV}{A}$$

N_o is the original concentration; N_t is the concentration at time t; V is the volume of the vessel being tested; and A is the surface area of the vessel which in this case is calculated on the assumption that the bladder approximates a sphere (3).

Hemotopic Bladder Permeability to Water and AAF. The ureters of anaesthetised control animals were catheterized for urinary diversion. The bladder was emptied through a urethral catheter, and then it was filled with THO-AAF stock solution (0.1 to 0.3 ml) to achieve a pressure of 5 to 6 cm of water.

A 0.05 ml aliquot taken with the same syringe was used as a standard to calculate the dose of each isotope instilled. At the end of the study period, the bladder neck was clamped and the bladder was excised, bisected, rinsed in 5 ml of 0.9% saline, and dissolved in 1 ml Soluene-100. The permeability coefficients were calculated as in the experimental group.

Water and AAF Permeability at Additional Time Intervals in Homotopic Bladders. Groups of six animals were studied at 0.25, 0.50, 2 and 6 hours. The techniques were the same as described for the 1 - and 4 - hours homotopic THO-AAF studies.

Effect of Reduced Specific Acitivty of Water and AAF on Permeabilities in Homotopic Bladders. Three animals in the 1- hour and three in the 4- hour group were studied as described above for the homotopic bladder group except that the stock solution was diluted 1:10 with 0.9% saline to reduce the specific activity of the isotopes.

Heterotopic Bladder Permeability to Sodium. Another group of 12 animals with heterotopic bladder preparations was used to study sodium permeability. The procedures were the same as described for THO-AAF studies up to the point of removing the bladder-reservoir system. In this study, the reservoir and bladder were removed intact. The reservoir with the connector clamped, was separated from the bladder which was discarded. The reservoir and contents were put in a vial and counted between two 5-inch NaI crystals (Series 600 Nuclear Scanner, Ratheon Co., Waltham, Mass.) (5).

Each animal was then placed in a 5-inch diameter plastic container equidistant between the two NaI crystals and the entire animal was counted. A 0.08 μCi aliquot of isotope was then injected intraperitoneally, allowed to equilibrate for at least 15 minutes and the animal was recounted as a means of internal standardization and to calculate the activity of the total initial dose. The initial bladder dose was calculated by subtracting the activity counted in the reservoir from the total dose. Permeability coefficients were calculated as above.

Homotopic Bladder Permeability to Sodium. Animals were then counted as above. A 0.05 μCi aliquot of sodium-22 was injected intraperitoneally for internal standardization and calculation of the activity of the initial dose. Permeability coefficients were calculated using Fick's law as above.

Calculations to Determine THO and AAF Permeability from 60 to 240 Minutes. In the cases of water and AAF the permeability coefficients were calculated for the time period 60 to 240 minutes in both heterotopic and homotopic bladders. This was done by using the permeability coefficients calculated from the observed data in the 1- hour studies to determine the amount of each label that would be absorbed during the first 60 minutes of the 4- hour studies. This amount was then subtracted from the N_o values for each animal and a calculated permeability coefficient was determined for the 60 to 240 minute time interval using Fick's law.

RESULTS

Table 1 shows the mean permeability coefficients and the standard deviations of each as calculated from observed data. The number of animals studied in each group is in parentheses. All transplanted bladder preparations were sterile as determined by culture.

In heterotopic bladders, the mean permeability coefficients for water and AAF were significantly lower ($p < .01$ and $p < .02$ respectively) when calculated from 4- hour studies as compared to 1- hour studies. In homotopic bladders, the values for water and AAF were again significantly lower ($p < .01$) when calculated from the 4- hour data compared to the 1- hour calculations. The permeability coefficients to AAF and water did not change significantly after 2 hours in the heterotopic bladders.

There was no significant difference ($p > .05$) in the permeability coefficients to sodium when calculated from the 1- and 4- hour data in either heterotopic or homotopic bladders. Also, there was no significant difference ($p > .05$) between the permeability coefficients of heterotopic bladders compared to homotopic bladders at either time period.

When the initial specific activity of the THO and AAF labels was reduced to 1/10 the original levels, there was no significant difference in the respective permeability coefficients when com-

Table 1. Mean permeability coefficients for water, AAF and sodium in the heterotopic and homotopic rat bladders expressed as mean ± S.D. x 10^{-6} cm sec^{-1} (Number of animals)

Time (hours)	P_{THO}		P_{AAF}		P_{Na}	
	Heterotopic	Homotopic	Heterotopic	Homotopic	Heterotopic	Homotopic
0.25	-	53.4 ± 19.1 (6)	-	183.2 ± 73.0 (6)	-	-
0.50	-	44.4 ± 15.1 (6)	-	106.6 ± 35.2 (6)	-	-
1	47.0 ± 9.3 (5)	47.3 ± 12.5 (7)	31.6 ± 9.7 (5)	54.2 ± 13.1 (7)	2.0 ± 0.6 (4)	2.3 ± 4 (6)
1 (1/10 activity)	-	33.7 ± 7.8 (3)	-	60.9 ± 11.6 (3)	-	-
2	-	37.8 ± 5.9	-	37.2 ± 14.0	-	-
4	27.5 ± 6.5 (6)	28.3 ± 3.7 (7)	18.7 ± 5.1 (6)	26.6 ± 2.3 (7)	3.2 ± 2.0 (3)	1.2 ± 0.9 (6)
4 (1/10 activity)	-	27.2 ± 2.0 (3)	-	26.2 ± 0.2 (3)	-	-
6	-	29.2 ± 15.0 (6)	-	22.9 ± 6.0 (6)	-	-

pared to those determined at the original specific activity of the labels.

Table 2 shows the calculated water (THO) and AAF permeability coefficients in heterotopic and homotopic bladders when the absorption for the first 60 minutes is mathematically calculated and substracted from the original amount introduced into the bladders. There was no significant difference ($p > .05$) between the AAF permeability coefficient of heterotopic and homotopic bladders. However, there was a 24% increase in the permeability coefficient for water in the heterotopic bladder when compared to the homotopic bladder ($p < .01$).

Table 2. Mean permeability coefficients for water and AAF from 60 to 240 minutes in heterotopic and homotopic rat bladders, expressed as mean ± S.D. x 10^{-6}cm sec^{-1}

	(Number of Animals)	
	P_{THO}	P_{AAF}
Heterotopic	20.7 ± 3.1 (6)	20.2 ± 6.2 (6)
Homotopic	16.7 ± 0.5 (7)	19.5 ± 0.5 (7)
P	< .01	> .5

DISCUSSION

Previous reports have shown that there was no net volume change during diffusion studies when isotonic solutions were used (1, 9). Therefore based on these data, all solutions used in these experiments were isotonic to eliminate volume changes during the studies. Also since published studies have shown that temperature and hydrostatic pressure directly affect permeability, all solutions were warmed to 37°C and instilled to a pressure of 5-6 cm of water which approximates bladder resting pressures (3, 4). The sodium studies were performed separately because the energy emission spectrum of sodium-22 overlaps the other two labels. In the sodium studies, the amount of label absorbed (total body count) was used because the amount absorbed during the study would be about 1-2% which would be of the same order of magnitude as the error in the recovery technique.

The permeability coefficients should be independent of time; however, in the initial studies, 1- hour data gave higher permeability coefficients than 4- hour data. To test the hypothesis that the

manipulation of the bladder at the initiation of each study causes a transient increase in permeability, four additional time periods were studied in the homotopic bladder. These data showed a transient increase in permeability initially with a large variation among animals. The bladders reached an apparent "steady state" after 1 hour. The standard deviations were small at the 4- hour interval. The larger standard deviations in the 6- hour group may represent instability of the animals after the prolonged period of anaesthesia. Therefore, the 4- hour studies are believed to be the most representative of the permeability coefficients as originally calculated. These data from the 4- hour studies were then recalculated by mathematically subtracting the absorption which would have occurred over the first 60 minutes while the system was stabilizing. As would be expected, these values from 60 - 240 minutes were slightly lower then the original 4- hour volume because the permeability is abnormally high during the stabilization period.

The permeability coefficient for sodium in homotopic bladders (1.2×10^{-6} cm sec^{-1}) agrees closely with a previously published study (1.3×10^{-6} cm sec^{-1}) (3). The permeability coefficient for water has not been previously reported for the rat bladder. However, the 16.7×10^{-6} cm sec^{-1} value for homotopic bladders is of the same order of magnitude as other mammalian bladders such as humans, dogs and rabbits ($60\text{-}76 \times 10^{-6}$cm sec^{-1}) (3). The lower permeability to water in the rat seems to be in keeping with its low sodium permeability compared to other mammals.

The permeability coefficients of the homotopic bladder to water and AAF were not significantly altered by reducing the specific activity to 1/10 the original level. These data confirm the accuracy of the isotope recovery and counting techniques over a wide range of activity levels.

The respective sodium and AAF permeability coefficients were not significantly different in heterotopic and homotopic bladders. Although there was a statistically significant increase in the permeability coefficient of the heterotopic bladder to water (24%), this translates to 46% absorption per hour in the heterotopic bladder compared to 47% in the homotopic bladder.

The above data indicate that the permeability characteristics of the transplanted or heterotopic bladder are similar to the homotopic bladder. These permeability studies and the previously described morphologic studies support the use of the heterotopic bladder as a model of a defunctionalized or diverted bladder.

Acknowledgements. This work was supported in part by U. S. Public Health Service Grant CA 14649 through the National Bladder Cancer Project and by Cancer Center Support Grant CA 15145 through the National Cancer Institute and the Northwestern University Cancer Center. Tobramycin was provided for this study by Eli Lilly Co., Inc., Indianapolis, Indiana. Nuclear scanning was performed by the Department of Nuclear Medicine, Northwestern Memorial Hospital, Chicago, Illinois.

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