Mice lacking the dopamine transporter display altered regulation of distal colonic motility

JULIA K. L. WALKER, RAUL R. GAINETDINOV, ALLEN W. MANGEL, MARC G. CARON, AND MICHAEL A. SHETZLINE
Howard Hughes Medical Institute, Departments of Cell Biology and Medicine, Division of Gastroenterology, Duke University Medical Center, Durham, North Carolina 27710

Received 3 December 1999; accepted in final form 15 March 2000

Walker, Julia K. L., Raul R. Gainetdinov, Allen W. Mangel, Marc G. Caron, and Michael A. Shetzline. Mice lacking the dopamine transporter display altered regulation of distal colonic motility. *Am J Physiol Gastrointest Liver Physiol* 279: G311–G318, 2000.—The mechanisms by which dopamine (DA) influences gastrointestinal (GI) tract motility are incompletely understood and complicated by tissue- and species-specific differences in dopaminergic function. To improve the understanding of DA action on GI motility, we used an organ tissue bath system to characterize motor function of distal colonic smooth muscle segments from wild-type and DA transporter knockout (DAT−/−) mice. In wild-type mice, combined blockade of D1 and D2 receptors resulted in significant increases in tone (62 ± 9%) and amplitude of spontaneous phasic contractions (167 ± 24%), and electric field stimulation (EFS)-induced (40 ± 8%) contractions, suggesting that endogenous DA is inhibitory to mouse distal colonic motility. The amplitudes of spontaneous phasic and EFS-induced contractions were lower in DAT−/− mice relative to wild-type mice. These differences were eliminated by combined D1 and D2 receptor blockade, indicating that the inhibitory effects of DA on distal colonic motility are potentiated in DAT−/− mice. Motility index was decreased in wild-type mice relative to wild-type mice. The fact that spontaneous phasic and EFS-induced contractile activity were altered by the lack of the DA transporter suggests an important role for endogenous DA in modulating motility of mouse distal colon.

Gastrointestinal (GI) motility is modulated by a complex system of intrinsic and extrinsic nerves, circulating hormones, and locally produced mediators. One such modulator is dopamine (DA). A description of the effect of DA on GI motility is complicated for three main reasons. First, DA can produce both inhibitory and excitatory effects on GI motility (reviewed in Ref. 27). Generally, the excitatory response, which is mediated by D2 or presynaptic receptors, occurs at a lower agonist concentration than the inhibitory response, which is mediated by D1 or postsynaptic receptors. Second, the mechanism of DA action and location of DA receptors are controversial. For example, studies using guinea pig stomach and rabbit ileum suggest that DA-mediated inhibition of GI motility occurs through modulation of the enteric nervous system (9, 25), whereas reports using gastric tissue from opossum indicate direct DA effects mediated by DA receptors on smooth muscle cells (3, 22). Third, conclusions regarding the role of DA in modulating GI tract motility have primarily been reached using pharmacological techniques, interpretation of which is confounded by the ability of DA agonists to activate adrenergic receptors (reviewed in Ref. 27). Thus more research is required to improve our understanding of DA-mediated regulation of GI motility.

To better understand the role that DA plays in regulating GI motility, we characterized DA-mediated regulation of distal colonic motility in wild-type mice and in mice lacking the DA transporter (DAT−/− mice). The DA transporter (DAT) regulates the concentration of DA in the synaptic cleft by rapidly removing the released neurotransmitter from the extracellular space (reviewed in Ref. 6). Recently, there has also been evidence demonstrating the presence of the DA transporter in DA-producing endocrine cells of the gut (11). Despite diminished intraneuronal levels of DA, extracellular DA levels are elevated and DAT−/− mice display a central hyperdopaminergic phenotype characterized by increased locomotion, neuroendocrine dysfunction, and dwarfism (8). We explored whether DAT−/− mice may also exhibit a hyperdopaminergic phenotype in peripheral tissue such as the distal colon. In the present report, the effects of DA and DA receptor antagonists on colonic contractility were evaluated in wild-type and DAT−/− mice.

Our results demonstrate that motility of the mouse distal colon is under basal inhibitory control by DA and that this effect is potentiated in mice lacking the DAT. This hyperdopaminergic phenotype associated with DAT−/− mice is characterized by an impaired ability to generate contractile force coupled with an increased frequency of spontaneous phasic contractions. The DAT−/− mouse could be an important model for further characterization of the role of DA in regulating GI motility.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
DOPAMINERGIC REGULATION OF GI MOTILITY

Krebs solution and bubbled continuously with a mixture of homozygous DAT

General Methods

METHODS

DAT knockout mice were created through genetic deletion of the DAT by homologous recombination (8). Wild-type and homozygous DAT −/− mice were derived from crossing heterozygous DAT C57BL6/129SvJ animals. Mice were housed in an animal care facility at a temperature of 23°C on a light-dark cycle of 12:12 h and were caged with approximately three littermates of the same sex, having free access to food and water. Animal care was in accordance with institutional guidelines. Mice 12–20 wk of age (mean 16 wk) were prepared for experiments. Mice were housed in an animal care facility at a temperature of 23°C on a light-dark cycle of 12:12 h and were caged with approximately three littermates of the same sex, having free access to food and water. Animal care was in accordance with institutional guidelines. Mice 12–20 wk of age (mean 16 wk) were prepared for experiments.

Distal colon preparations were allowed to equilibrate in Krebs solution for at least 60 min before being adjusted to optimal length. The mechanical activity of the longitudinal muscle was measured isometrically with a calibrated force displacement transducer (Radnoti) and acquired using a computer data acquisition program (CODAS, DATAQ). Acquired data was analyzed using peak detection software, and data were imported into a spreadsheet for calculation of all tension parameters.

A Grass S88 stimulator was used to produce electric field stimulation (EFS) of tissues by passing current pulses between ring electrodes. In all experiments, pulses of 0.1-ms duration, 40 V, and 5 Hz were applied for 10 s. These stimulus parameters were chosen on the basis of preliminary studies that demonstrated maximal contractile tension with little or no direct effect on smooth muscle, as assessed by the ability of 1 μM TTX to abolish the response.

Experimental Protocols

Dose response to DA. Increasing doses of DA were added, in half-log increments, to the organ tissue bath. With each new addition of DA, tissues were allowed to equilibrate for 2 min before EFS.

Effect of D1 and D2 receptor blockade. For antagonist dose-response protocols, tissues were incubated with 10 μM sulpiride and SCH-23390 for 8 min before EFS or addition of DA. If tissues were used for more than one protocol, a 30-min washout period was conducted between protocols.

Motility index. We used a modified equation to calculate motility index (2, 14). The motility index of phasic activity was calculated as the total area under the tension curve minus the area due to smooth muscle tone. Smooth muscle tone was calculated as the tension measured when no spontaneous phasic contraction was in progress. For each 2-min period (over the 30-min interval), each valley (minimum tension between spontaneous phasic contractions) was marked, sorted, and averaged. Each 2-min average tension was multiplied by 120 s, and these products were summed to give the area due to smooth muscle tone. Data used to calculate motility index were recorded before addition of test agents.

KCl depolarization. KCl was added to the Krebs solution with equimolar replacement of NaCl (18).

HPLC assessment of tissue content of DA. Dissected GI tissue was homogenized in 5 vol of 0.1 M HClO4 containing 50 ng/ml 3,4-dihydroxybenzylamine as an internal standard. Homogenates were centrifuged for 10 min at 10,000 g. Supernatants were filtered through a 0.22-μm filter and analyzed for levels of DA using HPLC with electrochemical detection as described previously (26). Monoamines and metabolites were separated on a microbore reverse-phase column (C-18, 5 μm, 1 × 150 mm; Unijet, Bioanalytical Systems) with a mobile phase consisting of 0.03 M citrate-phosphate buffer with 2.1 mM octyl sodium sulfate, 0.1 mM EDTA, 10 mM NaCl, and 17% methanol (pH 3.6) at a flow rate of 90 μl/min and detected by a 3-mm glass carbon electrode (Unijet) set at +0.65 V. The volume of injection was 5 μl.

Drugs and Chemicals

DA, sulpiride (D2 receptor antagonist), and Sch-23390 (D1 receptor antagonist) were purchased from Research Biochemicals. Carbachol and sodium nitroprusside (SNP) were purchased from Sigma Chemicals. Other chemicals used were of reagent grade.

Calculations and Statistics

The maximal contractile response to 100 μM carbachol was measured in each experiment. The amplitude of EFS-induced or spontaneous phasic contractions was calculated as the difference between peak tension developed and the average control baseline tension. Statistical analyses were carried out using the computer software package GraphPad Prism. To determine differences between mouse genotypes, a Student’s two-tailed t-test was used. To determine the effect of treatment within a mouse genotype, a Student’s two-tailed t-test for repeated measures was used. Results were considered significantly different if P < 0.05.

RESULTS

Maximal Contractile Response to Carbachol

The maximal contractile tonic tension generated in response to 100 μM carbachol was significantly less in DAT −/− mice (2,908 ± 141 mg) relative to wild-type mice (3,492 ± 140 mg) (P = 0.03).

Effect of DA on EFS-Induced Contractions in Wild-Type Mouse Distal Colon

To assess the effect of DA on distal colonic contractions, the amplitude of EFS-induced contraction was measured in the presence of increasing concentrations of DA. Figure 1 shows that DA inhibits the amplitude of EFS-induced contraction in a dose-dependent manner and that this effect is competitively antagonized by combined D1 and D2 receptor blockade. (EC50: intact, 4.5 × 10−6 M; D1 and D2 receptor blocked, 12.9 × 10−6 M). Pretreatment of tissues with D1 and D2 receptor blockers resulted in en-
The effect of D1 and D2 receptor blockade on basal smooth muscle tone and spontaneous phasic contractions (Fig. 2). These data suggest that EFS activates the release of DA, which acts at DA receptors to inhibit the amplitude of the resultant contraction. Thus DA, whether exogenously applied or endogenously released, acts at DA receptors to inhibit the amplitude of an EFS-induced contraction. Although EFS mimics a physiological event (depolarization of neurons), this does not necessarily implicate DA in the regulation of intrinsic distal colonic motility.

**Effect of DA on the Motility of Wild-Type Mouse Distal Colon**

To determine whether DA participates in the regulation of intrinsic distal colonic motility, we measured tonic and spontaneous phasic contractile activity before and after D1 and D2 receptor blockade. A representative recording (Fig. 3A) shows that smooth muscle tone and spontaneous phasic contraction amplitude are enhanced following D1 and D2 receptor blockade. Figure 3B illustrates the average effect of D1 and D2 receptor blockade on basal smooth muscle tone and spontaneous phasic contractions. Thus spontaneous phasic motility and tone of mouse distal colon are under basal inhibitory control by a DA-mediated system.

**Tissue DA Concentration in Mouse Distal Colon**

Tissue DA concentration primarily reflects intracellular DA stores (reviewed in Ref. 7) and was found to be significantly reduced in the distal and proximal colon of DAT −/− mice (Fig. 4). Previous studies have shown similar depletion of DA stores in central neurons of DAT −/− mice (15). These data support, albeit indirectly, the importance of DAT-related processes in these tissues and suggest that in DAT −/− mice DA is not efficiently cleared from the synaptic cleft, which may result in a hyperdopaminergic phenotype (6).

**Characterization of Distal Colonic Motility in the DAT −/− Mouse**

We hypothesized that the distal colon of DAT −/− mice would display functional hyperdopaminergia and that this condition would therefore manifest as decreased smooth muscle tone and diminished amplitude of spontaneous phasic and EFS-induced contractions relative to wild-type mice. Furthermore, we postulated that if these differences could be eliminated by D1 and D2 receptor blockade then this would provide further evidence that contractile differences observed between wild-type and DAT −/− mice are due to the pharmacological action of the enhanced dopaminergic condition. To assess the magnitude of distal colonic smooth muscle tone, we administered 100 μM SNP, a dose that we determined elicits maximal relaxation. SNP caused a significantly smaller relaxation in DAT −/− mice (366 ± 48 mg) than wild-type mice (499 ± 34). Furthermore, the amplitude of spontaneous phasic and EFS-induced contractions was significantly less in DAT −/− mice relative to wild-type mice (Fig. 5, A and B, left). Since DA inhibits the amplitude of spontaneous phasic and EFS-induced contractions in wild-type mice, these data are consistent with the hypothesis that DAT −/− mice display decreased distal colonic motility secondary to a state of hyperdopaminergia. The observation that differences in contraction amplitude were abrogated by D1 and D2 receptor blockade (Fig. 5, A and B, right) supports the notion that DA is responsible for the reduced contraction amplitude observed in DAT −/− mice.

**Motility Index of Wild-Type and DAT −/− Mouse Distal Colon**

To further evaluate the role of DA on GI motility, the motility index was measured in DAT −/− and wild-type mice. In DAT −/− mice, the average motility index (area under the tension curve minus the area due to basal tone) was significantly diminished relative to wild-type mice.

![Fig. 2. Effect of D1 and D2 receptor blockade on the amplitude of EFS-induced contraction in wild-type mouse distal colon. The amplitude of contraction was increased during D1 and D2 receptor blockade relative to control. Means ± SE are shown. *P < 0.05 vs. control.](http://appliedphysiology.org/10.2203/333)
wild-type mice (Fig. 6). To determine the principal parameter responsible for the decreased motility index in DAT\(^{2/-}\) mice, we calculated spontaneous phasic contraction frequency and amplitude. Although spontaneous phasic contractions were much more frequent in DAT\(^{2/-}\) mice than in wild-type mice (Fig. 7A), the distribution of the amplitude of these contractions is heavily weighted toward those of smaller amplitude (Fig. 7B).

To determine whether the difference in phasic contraction frequency was a function of a more depolarized membrane potential in DAT\(^{2/-}\) mice, tissues were incubated in modified Krebs solution containing increasing concentrations of K\(^+\). According to Mangel et al. (17) a greater increase in extracellular K\(^+\) is required to depolarize cells with an already depolarized resting membrane potential. In wild-type mice, 10–20 mM KCl significantly increased spontaneous phasic contraction frequency relative to control. These concentrations of KCl had no significant effect on spontaneous phasic contraction frequency in DAT\(^{2/-}\) mice, suggesting that the resting membrane potential in DAT\(^{2/-}\) distal colon is depolarized relative to wild-type mice (Fig. 7C). In both wild-type and DAT\(^{2/-}\) mice, incubation with KCl concentrations \(>20\) mM resulted in decreased spontaneous phasic contraction frequency (Fig. 7C).

**DISCUSSION**

In this study we demonstrated that DA-mediated inhibitory input is tonically supplied to the mouse colon. Furthermore, we showed that this inhibition is enhanced in the DAT\(^{-/-}\) mouse, which is consistent with a peripheral hyperdopaminergic phenotype. Finally, these studies provide evidence that endogenously released DA inhibits both spontaneous phasic and EFS-induced contraction amplitude.

Little is known about the role of DA in regulating GI motility in the mouse. Eaker et al. (4) provided evidence indicating the presence of DA-containing neurons in the myenteric plexus of the GI tract of mice; however, an earlier report by Fontaine et al. (5) reported that DA receptors were not present in the mouse colon. Our findings indicate a role for DA in regulating murine distal colon motility and highlight the importance of the DA transporter for this regulation.

This study demonstrates that DA inhibits distal colonic tone and the amplitude of spontaneous phasic and EFS-induced contractions in wild-type mice. This inhibitory effect of DA is more pronounced in DAT\(^{-/-}\) mice, suggesting that DAT\(^{-/-}\) mice display peripheral hyperdopaminergia. This “functional” excess of...
DA in the periphery is consistent with that previously described for the central nervous system of DAT −/− mice (6, 8). In central neurons, one of the hallmarks of this hyperdopaminergic phenotype is depletion of DA stores. We also found decreased DA stores in DAT −/− mouse distal colon.

In distal colonic smooth muscles, both spontaneous phasic and tonic components of tension are present (reviewed in Ref. 16). Spontaneous rhythmic or “phasic” smooth muscle cell contractions are initiated by slow waves. These slow waves depolarize the smooth muscle cell membrane potential, leading to opening

---

**Fig. 5.** Comparison of distal colonic phasic and EFS-induced contraction amplitude in DAT −/− mice and in age- and litter-matched wild-type mice with and without D1 and D2 receptor blockade. A: typical recordings of tension developed in wild-type (top trace) and DAT −/− (bottom trace) mouse distal colon under control conditions (left) and following D1 and D2 receptor blockade (right). Arrows indicate initiation of EFS. B: average amplitudes of phasic and EFS-induced contractions were greater in wild-type mice compared with DAT −/− mice during control conditions (left); however, average amplitudes were not different following D1 and D2 receptor blockade (right). Means ± SE are shown. *P < 0.05 for wild-type vs. DAT −/− mice.

**Fig. 6.** Motility index of mouse distal colon. A: typical recordings of phasic contractile activity in wild-type (top trace) and DAT −/− (bottom trace) mouse distal colon under control conditions. B: average motility index was greater in wild-type mice compared with DAT −/− mice. Means ± SE are shown. *P < 0.05 for wild-type vs. DAT −/− mice.
of voltage-sensitive Ca\(^{2+}\) channels with a subsequent influx of Ca\(^{2+}\) and, ultimately, contraction (reviewed in Ref. 10). The increased frequency of spontaneous phasic contractions observed in DAT \(^{-/-}\) mice, combined with their diminished response to KCl depolarization, would be consistent with the membrane potential of smooth muscle cells being depolarized in DAT \(^{-/-}\) mice relative to wild-type mice. Although transient depolarization of the membrane is usually associated with increased amplitude of contraction (due to opening of voltage-gated Ca\(^{2+}\) channels), the DAT \(^{-/-}\) mice exhibit decreased amplitude of contraction, as could occur with sustained depolarization at rest. However, to concretely determine whether or not the cell membrane potential is depolarized in DAT \(^{-/-}\) mice would require electrophysiological recording.

DAT \(^{-/-}\) mice display decreased distal colonic smooth muscle tone. We used SNP, a nitric oxide (NO) donor, to relax distal colonic smooth muscle and observed that DAT \(^{-/-}\) mice relax less than wild-type mice. There are at least two possible explanations for these results. First, according to Tare et al. (24), NO has a reduced capacity to relax smooth muscle when membrane potential is depolarized. As mentioned above, the results of the KCl depolarization protocol could suggest that membrane potential is depolarized in DAT \(^{-/-}\) mice relative to wild-type mice. Second, the reduced tone observed in DAT \(^{-/-}\) mice could be a result of interactions of DA with other steps in the process of pharmacomechanical coupling. For example, stimulation of G protein-coupled receptors, such as DA receptors, leads to activation of second messenger-dependent protein kinases (21). These kinases can affect smooth muscle tone by regulating voltage-sensitive Ca\(^{2+}\) channels, decreasing the sensitivity of contractile proteins to Ca\(^{2+}\), or altering the release/sequestration of sarcoplasmic reticulum Ca\(^{2+}\) stores (reviewed in Ref. 1).

Carbachol induces distal colonic smooth muscle contraction. In the present study, the amplitude of carbachol-induced contraction was significantly reduced in DAT \(^{-/-}\) mice relative to wild-type mice. Although this measure can be used to normalize differences in tissue cross-sectional area, values reported herein were not normalized. The data in Fig. 5 show that a reduction in cross-sectional area of the smooth muscle wall is not responsible for the reduced contraction amplitude observed in DAT \(^{-/-}\) mice. Rather, the results suggest that the tension developed by DAT \(^{-/-}\) mouse colon is similar to that of wild-type mice when D\(_1\) and D\(_2\) receptors are blocked, suggesting that DA is responsible for the reduced capacity to develop tension in DAT \(^{-/-}\) mice.

Although the mechanism of action of DA has not been fully characterized in the GI tract, it is generally accepted that in gut tissue D\(_1\) and D\(_2\) receptors are the primary functional subtypes of DA receptors (reviewed in Ref. 27). D\(_1\) receptors are mainly located postsynaptically, whereas D\(_2\) receptors are located pre- and postsynaptically. The use of receptor antagonists to discriminate DA effects from the effects of other catecholamines is not trivial and has led to some confusion regarding the physiological role of DA. There is some promiscuity among dopaminergic and adrenergic agonists/antagonists (reviewed in Ref. 27). Being aware of the controversy surrounding selectivity, or lack thereof, of DA receptor antagonists, we carefully chose and used agents that would simplify interpreta-
DOPAMINERGIC REGULATION OF GI MOTILITY

G317

tion of the data. Sch-23390 is a potent and selective D1 receptor antagonist that has very little effect on other receptors (12). Although D2 receptors and α2-adrenergic receptors are functionally similar, sulpiride (a D2 receptor antagonist) has a 200-fold greater affinity for D3 receptors than α2-adrenergic receptors (reviewed in Ref. 13). Thus Sch-23390 and sulpiride were used because they exhibit minimal nonspecific effects on other receptor systems.

Despite this selectivity, the relatively unimpressive effect of D1 and D2 blockade on the DA dose-response curve (Fig. 1) may lead one to conclude that Sch-23390 and sulpiride are poor receptor inhibitors or have nonspecific excitatory effects. The more likely explanation for the modest shift in the DA dose-response curve is nonspecific effects of DA at adrenergic receptors. Exogenously administered DA is known to activate adrenergic receptors, and if some fraction of the DA-mediated inhibition of EFS-induced contraction amplitude was mediated through adrenergic receptors, it would not be inhibited by the DA receptor antagonists used.

The data presented herein highlight the difficulty in trying to determine peripheral DA function by the addition of exogenous DA or its analogs. Through neuronal or autocrine release (4, 11, 20, 23), a high concentration of DA localizes at DA receptors in the GI tract, resulting in altered motility or secretion. This localized release of DA cannot be mimicked by exogenous addition of DA, which would have an ubiquitous distribution throughout the tissue. Thus exogenously administered DA may be of sufficient concentration to activate adrenergic receptors. Therefore, addition of exogenous DA is a less than optimal method of mimicking the physiological effects of DA in the GI tract.

The difficulties associated with adding exogenous DA to assess physiological function emphasize the importance of an animal model, such as the DAT-/- mouse, which is characterized by excessive endogenous DA. This mouse model provides an opportunity to study peripheral DA function simply by comparing the physiology of DAT-/- mice to wild-type mice.

Characterization of mouse physiology, and its relevance to that of humans, is of increasing importance given the current trend of linking genes to function. The DAT-/- mouse could be a valuable tool used to delineate the mechanisms of DA action on GI tract motility. Such studies may provide information useful in the delineation of therapeutic targets. Furthermore, studies investigating the role of DA in other peripheral systems may find the DAT-/- mouse to be an attractive model, since peripheral DA-mediated physiological effects can be revealed in these mice without exogenous administration of DA.

We thank Susan Suter and Jason Holt for animal care and genotyping.

M. G. Caron is an Investigator of the Howard Hughes Medical Institute. J. K. L. Walker is the recipient of an Medical Research Council/Canadian Lung Association Postdoctoral Fellowship. R. R. Gainetdinov is a visiting scientist from the Institute of Pharmacology, Russian Academy of Medical Sciences.

This work was supported in part by National Institute of Health Grants 5T32-DK-07568 (to M. A. Shetzline) and NS-19576, Bristol-Myers Squibb, and Zeneca Pharmaceuticals (M. G. Caron).

REFERENCES


