Computational Simulations of Dopaminergic Varicosities Suggest Two Sources of DOPAC Rather Than Two Populations of Dopamine Storage Vesicles

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Abstract: There are conflicting interpretations as to how many populations of dopamine storage vesicles exist in the nerve terminals of dopaminergic neurons. The goal of this project is to develop a computer simulation model of a dopaminergic varicosity that provides a plausible quantitative description of these populations and a possible set of rules for movement between two populations of vesicles. Both a one-compartment and a two-compartment model were devised for comparison, and it was found that the one-compartment model was able to successfully explain all of the data supporting two populations of storage vesicles in paradigms that stimulate dopaminergic neurons at rates much faster than physiological. However, these models could not explain other data supporting the two populations of vesicles concept. Models with two storage compartments were evaluated for ability to explain these data; however, none were successful. An alternate model was developed from the one-compartment model but with the addition that the dopamine synthetic process has a branch point where newly synthesized dopamine is either secreted into the extracellular space or converted to DOPAC, which is deposited into a cystolic compartment. This model successfully explains data regarding the specific activity of dopamine and metabolites after injection of labeled tyrosine into the varicosity, dopamine metabolite kinetics after inhibition of synthesis, and preferential secretion of newly synthesized dopamine, thus, suggesting that dopaminergic varicosities have two sources of DOPAC rather than two populations of dopamine storage vesicles.

Introduction

The dopamine terminal and synapse (shown in the figure to the left) has a vesicle (storage) compartment, a cytosolic compartment, an extracellular (synapse) compartment, enzymes to synthesize and degrade dopamine, and transporters to move dopamine between compartments. The dopamine transporter (DAT) moves dopamine molecules from the extracellular to the cytosolic compartment. The VMAT transporter moves dopamine from the cytosolic to the vesicular compartment. Exocytosis moves dopamine from the vesicular to the extracellular compartment. Dopamine is synthesized from the precursor tyrosine (shown in figure on the right) and is metabolized by the enzyme monoamine oxidase (MAO) to dihydroxyphenylacetic acid **(**DOPAC).

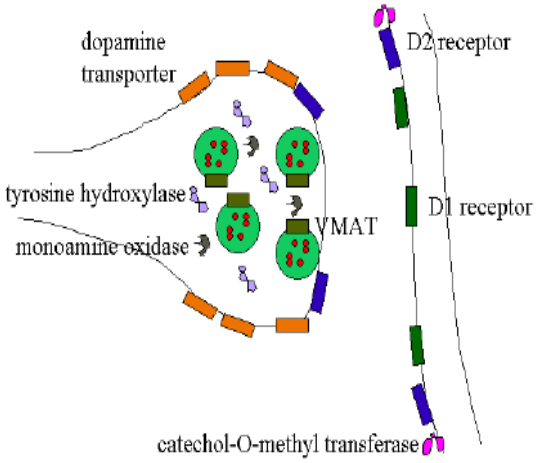

Figure 1: Illustrates the various processes that occur within the dopaminergic nerve terminal.

Figure 2: Illustrates the synthesis and metabolism of dopamine (DA).

Dopaminergic nerve signaling is involved in brain processing of pleasant rewarding experiences. Most of the dopamine molecules in the brain are made in advance and located in a storage compartment awaiting involvement in neural signaling. Dopamine is stored in small spheres called vesicles. Each dopamine nerve terminal contains about 200-300 vesicles (Pothos et al., 1998), each of which stores about 1500-2500 dopamine molecules (based on calculations from our lab group). Signaling events occur frequently (at a steady state, dopaminergic neurons fire at approximately 5 Hz (Bunney et al., 1973; Koeltzow et al., 1998)), rapidly moving vesicles into position to participate in an exocytoic event. It was thought that during signaling, vesicles fuse with the plasma membrane, releasing all of their contents into the extracellular space. This process is known as exocytosis. Recent evidence, however, has proposed a "kiss and run" mechanism, in which vesicles fuse with the membrane for a short period of time and then close soon thereafter to allow only a small portion of neurotransmitter into the synapse (Staal et al., 2004). Once in the extracellular space, dopamine can have one of three fates: it is lost in the extracellular space, converted into homovanillic acid, or taken back up into the presynaptic nerve terminal by the DAT transporter. Signaling is terminated primarily by the reuptake of dopamine by the DAT transporter. Drugs such as amphetamine and cocaine are shown to cause an excess of dopamine in the synapse by increasing synthesis, redistributing dopamine from vesicles to cytosol with subsequent transport by the dopamine transporter working in the reverse direction, and decreasing reuptake.

Currently, there are two hypotheses relative to dopamine storage. The one-compartment model maintains that each vesicle has an equal probability to participate in the next exocytotic event. The two-compartment model has a small portion of vesicles available to use in exocytosis and a large fraction held in reserve to be used in times of high demand for dopamine signaling.

Results from several experiments show five evidences for a two-compartment model. Five different experimental models were used: kinetics of dopamine loss after inhibition of dopamine synthesis (Javoy and Glowinski, 1971; Paden, 1979), specific activity of dopamine and

metabolites after inhibition of dopamine synthesis(Groppetti et al., 1977), kinetics of dopamine metabolite loss after inhibition of dopamine synthesis (Di Giulio et al., 1979), preferential secretion of newly synthesized dopamine (Besson et al., 1973), and impact of drugs on the amount of extracellular dopamine elicited by very fast firing conditions (Ewing et al., 1983). In the first four evidences, experimentally published measurements and our simulations were conducted under normal firing rates and covered a time duration of 30 to 70 minutes. The formation of dopamine metabolites is a slow process, and alterations in the rate of formation produce significant changes in metabolite levels. Therefore, under these conditions, both dopamine and metabolite levels can be studied. In the final evidence, which involved fast firing conditions, experimental published measurements and our simulations covered a time duration of up to 15 seconds. With such a short time, impacts on slow processes do not result in measureable changes. Therefore, metabolite levels were not examined in this study.

Originally, the goal of this project was to determine mathematical rules to account for dopamine storage into the two compartments proposed in these various experimental models. Once into the work, we discovered that some of the data did not seem compatible with a two-compartment model in our simulations. Therefore, we compared these five evidences with our one-compartment and two-compartment models to determine whether or not the data from the published literature can be explained solely on the presence of one-compartment for dopamine storage.

Methods

Schematics showing the compartments and movements between compartments are shown below for a one-compartment of vesicles model on the left and a two-compartment of vesicles model on the right. These models were used for the studies done under normal firing rate conditions. It is still unclear as to how dopamine molecules are transferred from vesicle to vesicle in the two-compartment model.

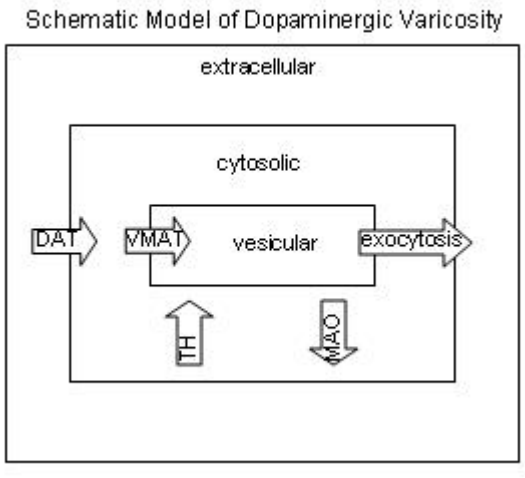

Figure: Schematic model of the dopaminergic varicosity in a one-compartment storage model.

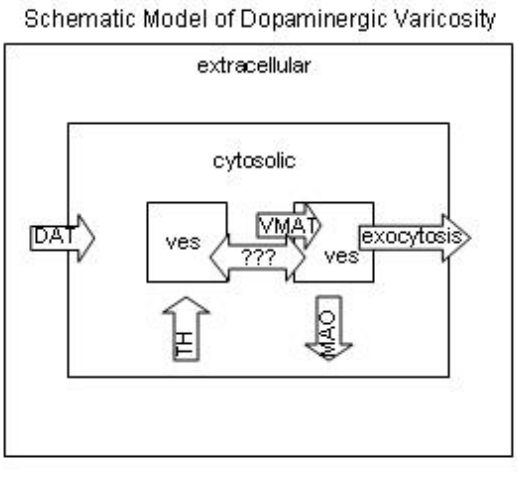

Figure: Schematic model of the dopaminergic varicosity in a two-compartment storage model.

The modeling process was accomplished using the following strategy:

1. Mathematical expressions were assigned to each process that occurs within the dopaminergic nerve terminal.
2. An initial value was assigned to dopamine in each of the three compartments (vesicular, cytostolic, and extracelluar).
3. The program calculates how much dopamine is added or subtracted to each of the compartments by TH, MAO, DAT, VMAT and exocytosis at various times (every 0.2 seconds).
4. The program computes a new value for dopamine in each of the three compartments based on the amount added and/or subtracted.
5. The amount of dopamine in each of the three compartment is compared to data from published literature
6. Adjustments were made in mathematical expressions and process rates until there was a good match between our data and the data from published literature.

Our strategy was to first run computer simulations using a one-compartment storage model, as it was the easiest to work with. If the one-compartment model could not explain the data from published literature, we would then run simulations using a two-compartment storage model. If that failed to explain the data from published literature, we would need to discuss further options.

The simulations of the fast firing experiment used a similar strategy with a different program. The following model shows the parameters used in the program.

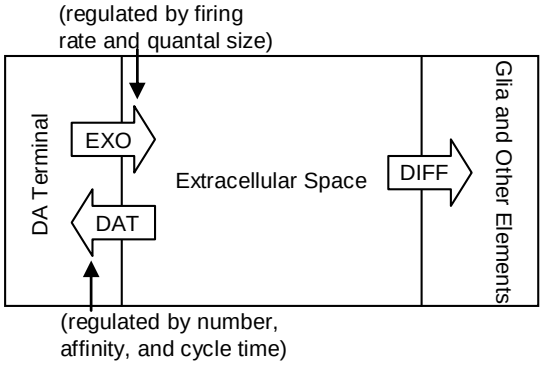

Figure: Schematic showing the parameters used in the simulation model. Abbreviations used are: DA, dopamine; DAT, dopamine transporter; EXO; exocytosis; DIFF, diffusion/uptake mediated by any process other than the dopamine transporter.

This model had a much shorter time-step in the program (0.0017 seconds) and a total simulated time duration of 15 seconds. Because of this short duration, only parameters that show significant changes within 15 seconds were included. The general strategy was the same as for the other model.

Results

Kinetics after alpha-methyl-p-tyrosine inhibition of synthesis

The rate of loss of dopamine from storage compartments was measured (data from CM Paden, J. Neurochem. 33:471-479:1979) or simulated as a function of time after inhibition of dopamine synthesis with alpha-methyl-p-tyrosine. The experimental data from Paden does not follow either a one-compartment or a two-compartment model (shown in the graph below), and therefore, cannot be interpreted as providing evidence for a two-compartment model. The experimental data, that is, the abrupt increase in dopamine just after 20 minutes, can only be explained by a transient loss of inhibitory effect of the alpha-methyl-p-tyrosine inhibitor of dopamine synthesis or a gain in ability to synthesize new dopamine by a process other than using the tyrosine hydroxylase enzyme.

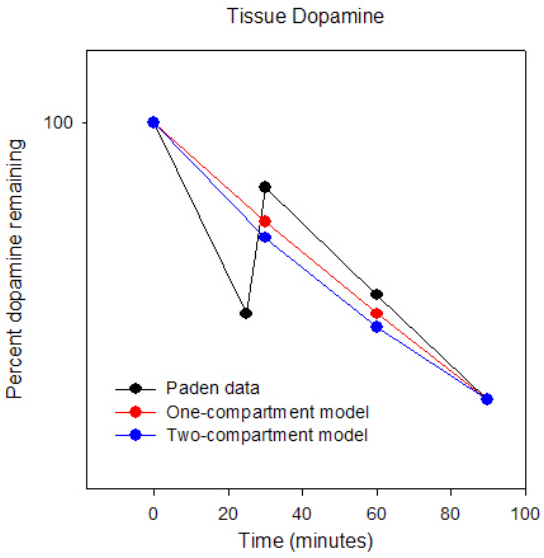

Figure: The abrupt increase just after 20 minutes does not follow either a one-compartment or a two-compartment model and can only be explained by a loss of inhibition of synthesis or a gain in the ability to synthesize dopamine by some other means.

Specific activity of dopamine and metabolites after the addition of labeled tyrosine

The experimental data (from Ewing et al., 1983; Groppetti et al., 1977) show that the ratio of DOPAC to dopamine specific activity is greater than one shortly after administration of radioactive tyrosine. Gropppetti argued that this is impossible in a one-compartment model storage system. We validated this arguement with our one-compartment model. We then evaluated two-compartment models with a variety of compartment sizes and rate of movement of dopamine between compartments. However, none of these simulations with our two-compartment models could reproduce the data. We concluded that a two-compartment model would not be able to explain the data from the published literature. We then looked to see what mathematical expressions were capable of explaining the published data. From these findings, we developed a different model with two sources of DOPAC synthesis, which is capable of reproducing the data. The graph on the left shows the model that we used. There are two sub-cellular compartments somewhere within the cytosol. One is the traditional vesicular compartment. The other is termed "mystery" compartment. Tyrosine hydroxylase is associated

with the mystery compartment, and all newly synthesized dopamine goes into this compartment. A fraction of the newly synthesized dopamine is secreted directly into the extracellular compartment, a fraction is metabolized to DOPAC, and a fraction remains in the compartment. The cytosolic dopamine metabolized by MAO goes directly into the mystery compartment. All other aspects of the model retain traditional, classical parameters. In the graph on the right, the blue line shows the simulations output that we achieved through this model. We varied values of the MAO_k in the two compartments, arriving at a value of MAO_k in the mystery compartment of 0.2 and MAO_k in the cytosolic compartment of 0.0044. Our model has a steady state level of 8.57 dopamine molecules in the mystery compartment. Thus, the amount of dopamine in the "mystery compartment" must be very low, about 8-9 dopamine molecules, compared to the 470,000 molecules in the vesicular compartment in the model. The red stars represent the results from the Groppetti et al. experiment. It can be observed that our data poses a very good fit to the data in the Groppetti experiment.

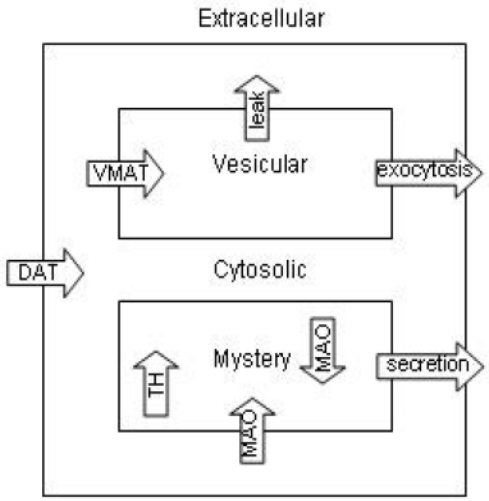

Figure: Schematic model of the dopaminergic varicosity in a one-compartment of storage vesicles model with two sources of DOPAC.

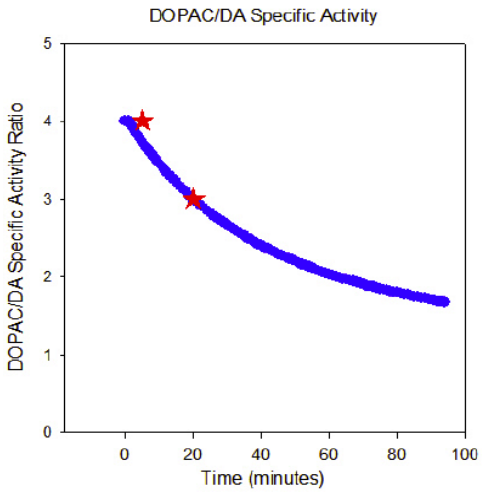

Figure: Our data closely match the data obtained from the Groppetti experiment. The argued that these results could only occur in a two-compartment model, however, we verified that our one-compartment model with two sources of DOPAC could reproduce similar results.

Dopamine metabolite kinetics after alpha-methyl-p-tyrosine inhibition of synthesis

The experimental data (from AM DiGiulio et al., 1979) show that the dopamine metabolite, DOPAC, disappears faster than does dopamine after inhibition of dopamine synthesis with alpha-methyl-p-tyrosine. The graph below shows dopamine and DOPAC loss from the straitum after inhibition of dopamine synthesis from the Giulio paper compared to expected loss from our traditional one-compartment model and our new model with two sources of DOPAC. The DiGiulio data show that seventy minutes after inhibition of synthesis, DOPAC was 42% of control and dopamine was 68% of control. For our classical one-compartment model, DOPAC and dopamine values for DOPAC and dopamine at t =70 minutes were 83% and 76%, respectively, showing a rate of dopamine and DOPAC loss to be very similar. This clearly did not match the pattern observed in the paper. Our new one-compartment model, however, had DOPAC and dopamine values of 66% and 82% of control, respectively. Our new model showed

the rate of DOPAC loss to be greater than that of dopamine loss, which did match their findings. The DiGiulio paper also showed more radioactive DOPAC than dopamine after synthesis was substantially inhibited. Therefore, we also ran similar calculations for radioactive dopamine and DOPAC. For these stimulations, we only inhibited synthesis 90%. When radioactive synthesis began it accounted for half of total synthesis. For both models, both DOPAC and dopamine were 10% of control, which did not match the pattern shown in the paper. At this time, we have no explanation for the Di Giulio results and do not have model that can match them.

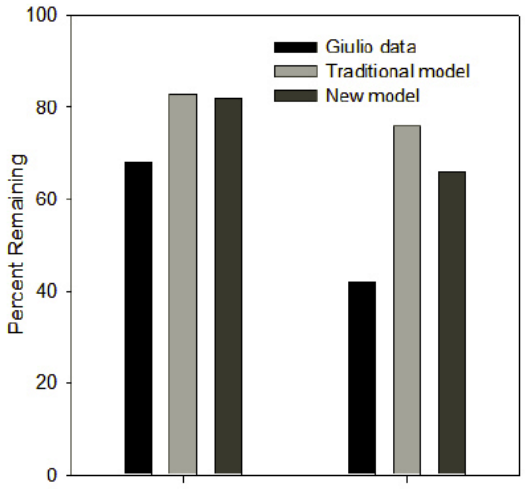

Figure: While our traditional one-compartment model could not reproduce the Giulio data, our one-compartment model with two sources of DOPAC did show the rate of DOPAC loss to be greater than that of dopamine loss; however, this difference was not as profound as that illustrated in the Giulio data.

Preferential secretion on newly synthesized dopamine

The experimental data (from Besson et al., 1973) involve measuring radioactive dopamine in fluid perfused over the surface of the striatum after radioactive tyrosine is added to the perfusate. When tritiated tyrosine was added to the perfusate, tritiated dopamine was recovered. When dopamine synthesis was inhibited, there was a gradual decrease in the amount of tritiated dopamine recovered. This set of data was interpreted as evidence for preferential release of newly synthesized dopamine. We could not reproduce these results with simulations using either a one-compartment or a two-compartment dopamine storage model. We reasoned that the results might be explained if the amount of dopamine collected to do the measurements is greater than the synthetic capacity of the tissue to replace the lost dopamine. We tested this hypothesis. We modified our program slightly to include a loss of dopamine from the extracellular compartment and an increase in the number of program iterations so that we could match the time course presented in the Besson paper. We set tyrosine hydroxylase activity at the value used for the "fast-state" of the enzyme in our no-fire project, 120 molecules per minute (24 per program iteration as compared to the control of 6 per program iteration). We set the rate of extraction of dopamine from the extracellular compartment at a continuous 5%. The graph below shows the comparison of our traditional one-compartment model and our new model with two sources of DOPAC with the Besson data. In these simulations, 5% of the dopamine was continuously removed from the extracellular compartment. Both models provide a reasonable match to the experimental data.

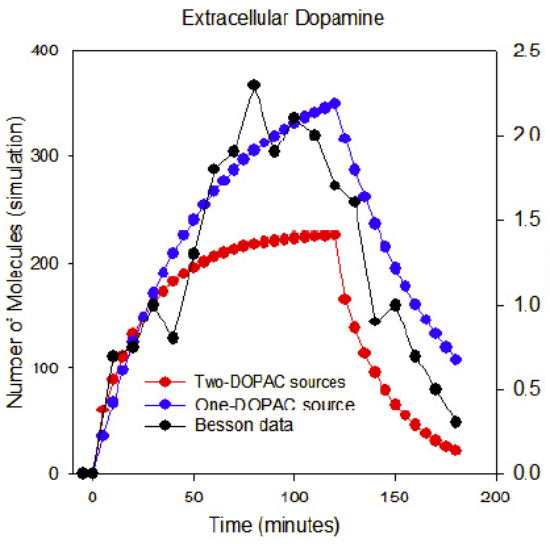

Figure: We accounted for a continuous loss of 5% for the rate of extraction of dopamine. We were able to reproduce the data with both our traditional onc-compartment model and our one-compartment model with two sources of DOPAC

Impact of drugs on the amount of extracellular dopamine elicited by very fast firing conditions

The experimental data (from AG Ewing et al., 1983) derive from measurements of extracellular dopamine in striatum when the cell bodies are electrically driven trains of 600 pulses over 10 seconds repeated every 20 minutes. In control animals, the amount of extracellular dopamine elicited by the pulses remains constant during the entire experiment. In animals where dopamine synthesis is inhibited, the amount of extracellular dopamine elicited by successive trains of pulses declines with time, reaching 80% decrement when total tissue dopamine (measure of storage) has only declined half this amount. We developed a simulation model for this experiment and determined how many dopamine molecules must be secreted after each pulse in order to produce the reported extracellular dopamine levels. We then compared these values to reported values for both extracellular and total tissue dopamine. The simulations show that the number of dopamine molecules secreted with each stimulation is proportional to the total tissue dopamine. This result exactly matches the expectation of a one-compartment dopamine storage model. The simulations also show that extracellular dopamine in this experimental paradigm decreases much faster after dopamine synthesis is stopped because of a non-linear relationship between the quantal unit and resultant amount of extracellular dopamine.

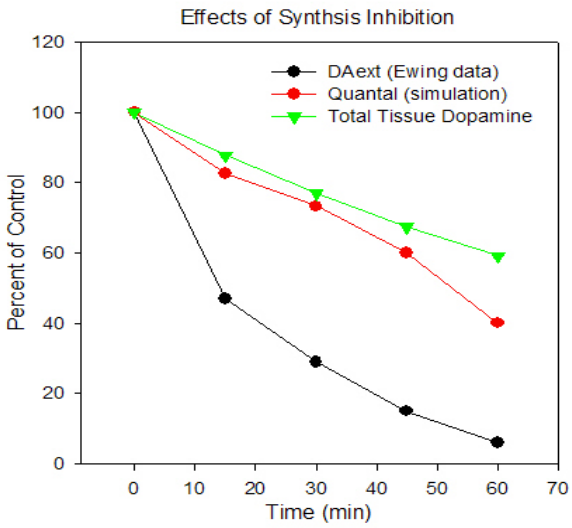

Figure: Our simulations show that the number of dopamine molecules secreted with each stimulation is proportional to the total tissue dopamine. This result exactly matches the expectation of a one-compartment dopamine storage model.

Discussion

The first four evidences we compared were all performed under normal firing conditions. Under these conditions, the one-compartment model seldom worked. This supported the interpretations in the published literature. In these papers, the existence of a two-compartment model was proposed, but no testing of this hypothesis was completed. Instead, they merely claimed that because the data could not be explained by a one-compartment model, it must be a two-compartment model. Therefore, our two-compartmental simulations became the first test of the two-compartment model. However, when simulations were run using our two-compartment model, it did not match the published data either. In light of this, we developed a new model with two DOPAC compartments and found that it could explain all of the data from published literature.

The two DOPAC compartments is a novel theory. The questions arises as to what evidences might be compatible with this model. First, one study on regulation of dopamine metabolism, has proposed a similar idea (Carboni et al., 1992). Second, other modeling projects have found that the standard model could not explain published data, but the two compartments of DOPAC model can. These projects, done by other students, are looking at the effects of antipsychotic drugs on total tissue DOPAC (project by Celia Fenell and Molly Bonfiglio) and at the effects of amphetamine on extracellular DOPAC (project by Heather Russell).

The last evidence, impact of drugs on the amount of extracellular dopamine elicited by very fast firing rate, was performed under very fast firing conditions. A one-compartment model was able to successfully explain the data from published literature. The major argument for two-compartments assumed a linear relation between the amount of dopamine involved in an exocytotic event and the amount of extracellular dopamine. Our simulations show that this is not a linear relationship under fast firing conditions. The reason is that the rate limiting step in dopamine removal from extracellular space changes from rate of binding to the transporter under normal firing conditions to the rate at which the transporter completes a transport cycle under

fast firing conditions. The fast firing simulations provide no data relative to the new two DOPAC compartments concept. The reason is that DOPAC is not even considered in this set of simulations because changes in DOPAC are slow and the simulation period is short (approximately 15 seconds).

Conclusion

The mechanism by which dopamine is stored, transferred, and released in the nerve terminals of dopaminergic neurons remains a question of controversy. There is much evidence for both a one-compartment and a two-compartment storage theory. In a one-compartment model, each vesicle has an equal probability to participate in the next signaling event. In a two-compartment model, a small portion of vesicles is available for use in the next signaling event, while a larger fraction is held in a reserve pool, only to be used in times of high demand. We found numerous papers that presented several arguments for a two-compartment model. We set out to determine mathematical rules to account for dopamine storage into the two compartments proposed in these studies. We looked at data using five different experimental paradigms: kinetics of dopamine loss after inhibition of dopamine synthesis, specific activity of dopamine and metabolites after the addition of labeled tyrosine, kinetics of dopamine metabolite loss after inhibition of dopamine synthesis, preferential secretion of newly synthesized dopamine, and the impact of drugs on the amount of extracellular dopamine elicited by very fast firing conditions. In doing this, we found that a two-compartment model could not explain all of the published data that have been interpreted as evidence for a two-storage compartment model. However, a model with one dopamine storage compartment with two sources of DOPAC was able to explain all of the published data. We propose that the two sources of DOPAC are from 1) synthesis using monoamine oxidase enzyme located in the mitochondria (well documented) and 2) synthesis in a multi-protein complex containing tyrosine hydroxylase and some form of monoamine oxidase. In this complex, newly synthesized dopamine can either be released into the cytosol or be further processed by monoamine oxidase to become DOPAC. These results suggest that DOPAC might be a signaling molecule. Other evidence for this hypothesis include: DOPAC levels in extracellular (signaling) compartment are much higher than dopamine levels and DOPAC levels in the cytosol (Rodriguez et al., 2007) and extracellular compartments are changed in interesting ways by a variety of drugs (Bardgett et al., 1997; Hurd and Ungerstedt, 1989; O'Dell et al., 1993).

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